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THE INFLUENCE OF PHYSICAL FACTORS ON THE VIABILITY OF SPORIDIA OF CRONARTIUM RIBICOLA FISCHER [1]

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INTRODUCTION

For years members of the staff of the Office of Forest Pathology of the Bureau of Plant Industry, United States Department of Agriculture, and others have attempted with varying degrees of success to artificially infect pines with the sporidia of various species of Cronartium. Efforts to infect white pines with the sporidia of *Cronartium ribicola* Fischer have met with discouraging lack of success until the last few years. Various investigators (*15*, *16*, *17*, *35*, *46*, *47*, *48*, *58*, *63*)[3] have scored successes in this direction, but the problem is so complicated that comparatively little is known about the physical conditions necessary for the successful artificial infection of white pines by this fungus. There are three distinct phases of the problem: (1) The factors controlling the formation, germination, and viability of the sporidia; (2) the conditions existing within the white pines at the time of inoculation; and (3) the physical conditions of the surrounding air at the time of inoculation. A preliminary study of the latter phase was made by York and Snell (*63*), who infected young plants of *Pinus strobus* L. at certain known relative humidities and temperatures of the surrounding air.

In 1923 the problem of studying the sporidia of *Cronartium ribicola* was assigned to the writers, but because of poor material that season's work was preliminary in nature. The work was continued in 1924. This paper presents the data obtained in both years.

HISTORICAL REVIEW

One can hardly appreciate the status of the knowledge on the germination of the teliospores, and of the formation and germination of the sporidia of *Cronartium ribicola*, without a historical survey of the literature on the genus Cronartium. Articles on other rust spores are also important as indicating possible methods of investigating the sporidia of *C. ribicola*. Within certain limits, facts which are true of other spore forms are likely to be true of sporidia. The morphology and cytology of the Uredinales in general have been investigated as thoroughly as those of any other fungus group. Yet Colley's paper (*18*) on the morphology and cytology of *C. ribicola*,

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[3] Reference is made by number (italic) to "Literature cited," p. 430.

published in 1918, seems to be the most complete study that has been made of any species of Cronartium. A consideration of the physiology of the germination of spores shows that in spite of much work there is still great lack of continuity in the results. Ward (*61*) said in 1902: "It is astonishing how little seems to have been done in ascertaining the conditions of germination of the uredospores." It seems to the writers, however, that the germination of uredospores has been more thoroughly investigated than that of any other form of rust spore. The following brief historical summary is arranged chronologically, to show clearly each investigator's contribution of new knowledge.

Unger (*60*) gave figures of the telia of *Cronartium asclepiadeum* (Wallr.) Fr., and of early stages of formation of the promycelia by germ tubes from teliospores in the columella.

The Tulasne brothers (*59*) also studied *Cronartium asclepiadeum*. They found that there was no ostiole in the telial column as was apparently generally supposed at that time. They also illustrated the germination of the terminal teliospores of the column, the 4-celled promycelia, the formation of sporidia by the promycelia, and the germination of the sporidia.

Bary (*8*) supplemented the Tulasne brothers' results by studies with other genera of the rusts. He made the following significant statements concerning the infection of hosts by the rusts:

> "There is no reason for admitting that a predisposition of the host to a certain disease determines its invasion by the parasite. On the contrary, the healthier the plant the more the parasite prospers, provided external conditions are favorable for its vegetation."

Reess (*41*) noted the formation of secondary sporidia by *Chrysomyxa abietis* (Wallr.) Unger.

Cramer (*19*) mentioned the breaking up of the promycelium of Gymnosporangium into conidiumlike bodies.

Bary (*9*) made the following statements concerning the forcible ejection of sporidia from the sterigmata of *Aecidium abietinum* Wallr.

> "Die Sporidien werden nach ihrer Ausbildung von den Sterigmen abgegliedert und eine kurze Strecke weit weggeschleudert; letzteres wohl durch den gleichen—meinerseits nicht untersuchten—Mechanismus wie die Sporen von *Coprinus*. Die Kraft, mit welcher sie geworfen werden, ist nicht gross."

Farlow (*27*), in the first detailed American study of a rust, described the breaking up of the promycelia of Gymnosporangia into conidiumlike bodies when they are suddenly dried.

Barclay (*1*), in a study of the new species *Aecidium strobilanthis*, noted that when germination of the teliospores took place at high temperatures, larger sporidia were produced than when germination was slow from the effect of low temperatures. Later (*2*) he recorded the breaking up of the promycelium into conidiumlike bodies in *Uromyces solidaginis* Niessl and *Caeoma smilacis* Barclay, and the germination of these anomalous conidia. He attributed the breaking up of the promycelia to the absence of air. He also observed (*3*) the throwing off of the sporidia of *Chrysomyxa himalense* Barclay from the sterigmata. He noted (*4*) the breaking up of the promycelia of *Gymnosporangium cunninghamianum* Barclay and the formation of secondary sporidia. He described (*5*) the method of germinating rust spores by floating them on water, rather than immersing them in it. He also stated (*7*) that the secondary sporidia formed by *Puccinia*

jasmini-chrysopogonis Barclay are about two-thirds as large as the primary ones, but that those of *Uromyces cunninghamianus* Barclay (*5*) were of approximately the same size as the primary ones.

Zalewski (*64*) studied the throwing off of spores by various Hymenomycetes. He also stated that the aeciospores of the rusts are thrown off forcibly to a distance of "10–15 mm." This observed distance appears to be an error due to convection air currents.

Kienitz-Gerloff (*33*) noted germ tubes of teliospores of *Gymnosporangium clavariaeforme* (Jacq.) DC. breaking up into conidium-like bodies, and the production of several germ tubes from one teliospore.

Plowright (*38*) compiled the knowledge of the British Uredineae. He stated that the longevity of the spores is influenced by the temperature and the dryness of the air, that the sporidia form germ tubes with somewhat pointed tips, and that sporidia may form secondary sporidia if germinated in water.

Carleton (*13*) studied the influence of various chemicals on the germination of rust spores.

Rauch (*39*) studied, among other fungi, three different genera of rusts. He found that the germination of freshly collected spores was influenced by ripeness, humidity, and temperature, but not by light. He also observed that a few days of drying lengthened the time necessary for germination, and that aeciospores and uredospores soon lost viability, but that teliospores retained it for several months after drying.

Sappin-Trouffy (*43*) reviewed the morphology of the Uredinales. For the genus Cronartium he gave figures of teliospores germinating at the tip of the telial column; of a teliospore immersed in water germinating and forming a four-celled structure, each cell of which produced a germ tube but no sporidium; and of the formation of secondary sporidia. He thus confirmed the morphological facts reported by earlier investigators.

Eriksson (*25*, *26*) investigated the effect of cooling on the subsequent germination of various rust spores, including aeciospores of *Cronartium ribicola*. He decided that such precooling accelerated and increased germination. He inclined to the idea that spores wet by rain just before using reacted to the cooling, while those which were not so moistened did not. He plainly described germination of the spores by floating them upon the surface of water in relatively large containers, in contrast with the usual drop-culture method.

Ward (*62*), working with *Puccinia dispersa* Eriksson, found that external and internal physical factors greatly influenced the distribution and persistence of a rust in its uredo stage. He recognized the effect of maturity on the germination of the uredospores, and the capriciousness of their germination, due to unknown causes. He found that uredospores remained viable in diffused sunlight from 30 to 61 days.

Carleton (*14*), after extended studies of various rusts, especially the grain rusts, stated that rust spores germinated readily in water; that under ordinary conditions the usual amount of growth was reached in an average of 24 hours; and that all growth stopped uniformly at a certain stage, even in the most carefully sterilized cultures.

Blackman (*10*) investigated the germination of teliospores of the Uredineae and the formation of the sporidia. He brought out clearly the fact that the sporidia are promptly formed in air, but not when the germ tube of the promycelial cell is immersed in water. Later

(*11*) he concluded that the teliospore is a spore mother cell which undergoes a tetrad division to form the four sporidia.

Klebahn (*34*) compiled the knowledge of the heteroecious Uredineae, adding much as a result of his own investigations. He clearly stated the fact that the sporidia are forcibly thrown from the sterigmata, but he did not include the genus Cronartium among the rusts in which this phenomenon occurs. He also argued that the spores are carried some distance by wind, and gave convincing proof that this actually occurs with certain species of the Uredineae.

Schaffnit (*44*) investigated the factors controlling the germination of the aeciospores and uredospores of the wheat rusts. He concluded that they mature only when attached to sporophores, and that moisture favors their production and germination.

Maire (*36*) summarized the known biology of the rusts. He emphasized that telia formed as a result of the decadence of the leaf or that part of the leaf upon which the sori were located. He reported that sporidia were thrown off forcibly, and that convection air currents picked them up and bore them off to indefinite distances; and that the number of sporidia produced and set free at a given center had a direct influence upon the distance that they might be carried and still infect their hosts.

Dietel (*21*) investigated the discharge of the sporidia of a number of the rusts, including species of Puccinia, Coleosporium, and Cronartium. However, he conducted only one experiment with Cronartium and it was unsuccessful, so this genus still remained to be investigated. He found in the other two genera that the sporidia were thrown off.

Robinson (*42*) studied the effect of external stimuli on the sporidia of *Puccinia malvacearum* Mont. In 12 hours he got good crops of sporidia, which germinated normally in proximity to leaves of numerous species of plants, and abnormally when near a few specific kinds. He found that the germ tubes were negatively heliotropic and were not attracted by small pieces of leaves in the cultures.

Reed and Crabill (*40*) made a detailed study of the germination of the teliospores of *Gymnosporangium juniperi-virginianae* Schweinitz, giving especial attention to factors which might influence their functions. They found that the teliospores on the outside of the spore tentacle germinated first and shriveled away, exposing those beneath, which germinated in turn. They determined the cardinal temperatures necessary for germination, that the presence of free water was required, and that carbon dioxide inhibited germination. They found that sporidia germinated by producing simple germ tubes or by forming secondary sporidia, which might germinate in the same manner as the primary onces. They observed that secondary sporidia were produced only when the primary sporidia were kept moist constantly from the time of production until germination. They ascertained that the longevity of the sporidia was five or six days in an air-dried condition; that drying was fatal to them, even at relatively low temperatures; and that in direct sunlight they were killed in two to five hours.

Colley (*18*) made a careful study of the anatomy and cytology of all the spore forms of *Cronartium ribicola*. He found that telial columns arose either from old uredinia or as entirely new and separate entities; that mature columns were aggregations of vertical

rows of mature teliospores; that the terminal spores of the column were the older ones, new ones being added by cell division at the base of the column; that all of the spores of a column might germinate in situ; that germ tubes from spores within the column pushed forth between the overlying rows of teliospores and then produced promycelia in the normal manner; that germ tubes of teliospores soon formed promycelia with four fertile cells, if not shut off from the air by immersion in water, in which case long germ tubes were formed, but no promycelia; that each fertile cell of the promycelium in turn produced a short germ tube or sterigma, from the tip of which a spherical sporidium was abstricted; that the sporidia germinated by producing relatively stout germ tubes; and that secondary sporidia were formed quite commonly.

Doran (*23*) determined the cardinal temperatures for the germination of the spores of several of the Uredinales, including the aeciospores and uredospores of *Cronartium ribicola*. He also (*24*) studied the factors controlling the functions of fungous spores in general. He found that mature spores endured a wider range of environmental conditions than did immature spores; that freshly matured spores were more viable than old ones; that the longevity of spores depended upon the conditions under which they were stored; and that crowding of spores in cultures inhibited germination.

Clinton and McCormick (*15*, *16*, *17*) studied the artificial infection of young white pines by *Cronartium ribicola*. They found that infection took place through the stomata of the leaves, and they traced the development of the mycelium in the host and its course down the leaf and into the bark.

Tubeuf (58) had previously found that infection of white pines by *Cronartium ribicola* took place through the leaves, but he had not so carefully determined the course of events within the leaves.

York (*49*, *50*, *51*)[4] made preliminary studies on the longevity of the sporidia of *Cronartium ribicola*. His results indicated that they are relatively short-lived and susceptible to external influences. He developed methods for obtaining abundant sporidia at will.

Spaulding (51) summarized the known facts concerning *Cronartium ribicola*, adding much as the results of the investigations by himself and associates.

Taylor (56) estimated the sporidial production of *Cronartium ribicola* in maximum infections on the various species of Ribes of the eastern United States.

York and Snell (*63*) found that sporidia of *Cronartium ribicola* infected young plants of *Pinus strobus* when the temperature was 64 to 65° F. and the relative humidity was 94 to 95 per cent in the inoculation chamber.

Spaulding (*52*) found teliospores of *Cronartium ribicola* germinating naturally out of doors in winter and producing sporidia in the snow. He also found that teliospores might retain viability until early winter when kept out of doors.

Buller (*12*) investigated the setting free of the sporidia of many of the Uredinales. He found in all species studied, that a tiny droplet of water formed at the base of the sporidium just at maturity,

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YORK, H. H., SNELL, W. H., OVERHOLTS, L. O., TAYLOR, M. W., and RATHBUN-GRAVATT, A. OBSERVATIONS ON THE SPORES OF CRONATIUM RIBICOLA FISCHER. [Unpublished manuscript.]

and that the sporidium was then shot off into space. He erred in attributing to Klebahn (*34*), the first mention of throwing off of sporidia of the rusts as it had been noted by earlier writers (*2*, *3*, *9*, *64*). He also erred in saying that Dietel (*21*) definitely proved that the sporidia of the genus Cronartium were thrown off from the sterigmata, for Dietel made only one test with a Cronartium and it was unsuccessful. There is little doubt that the sporidia of the Cronartiums are forcibly thrown off, but apparently this has not yet been proved.

Spaulding and Rathbun-Gravatt (*52*, *54*) studied the factors controlling the activities of the teliospores, and to some extent those of the uredospores, of *Cronartium ribicola*. They found that the teliospores might survive exposure out of doors for about three months; but that the uredospores survived a shorter time, two months after telia were formed upon the same leaves being about the limit for them.

Snell and Rathbun-Gravatt (*46*) successfully infected old white pines with sporidia of *Cronartium ribicola*, both with and without moist chambers. They conducted experiments only at high relative humidities and rather low temperatures.

This historical review indicates that knowledge of the morphology and cytology of Cronartium, particularly *C. ribicola*, is quite complete. While much study has been made of the influence of physical factors on the germination of spores of the Uredinales in general, it can not be said that the present knowledge on this subject is at all complete. There is now only a meager knowledge of the physical factors controlling the functions of the sporidia of any of the rusts. The physiological factors controlling the functions of the sporidia of Cronartium had been largely ignored until the problem of the factors controlling infection of pines was attacked.

MATERIAL NECESSARY AND AVAILABLE

Investigations of the type reported in this paper require an enormous number of sporidia, procurable only from a great number of telial columns, which in turn can usually be obtained only from very large numbers of telium-bearing Ribes leaves. Such masses of material can be collected only from Ribes bushes growing under the best conditions out of doors and subjected to heavy infection naturally. It is necessary to search over large areas to find enough Ribes bushes of the native wild species which fulfill all of these conditions. The different species of Ribes vary much in the number of telia and sporidia which they produce. Taylor (*56*) gives a graphic representation of the variation in telial production. The Ribes species also vary much in the typical size of leaves, and in percentage of total leaf surface which bears telia. *Ribes nigrum* L. which produces by far the most abundant supply of telia, was the mainstay in these investigations. Two dozen heavily infected leaves of this species often furnished enough material for a group of experiments consisting of 24 slides, the minimum number required for each day. To obtain a similar number of telia from *R. rotundifolium* Michx., a small-leaved species, more than 600 leaves were used. These were infected more heavily than this species usually is, and they were practically the entire season's crop in an especially good spot.

The period of natural production of telia varies with the season, (*51*) but for the northeastern States it extends from approximately July first until the Ribes leaves fall from the bushes. In 1923 investigations were conducted at Warrensburg, N. Y., which is located in the region of general white pine blister-rust infection, from August 1, when telia were first produced in abundance, until October 10. In many of these tests in 1923 it was necessary to use telia from stored dry leaves. In 1924 the investigations were continued at the same place from August 4 to September 10, when wet weather had germinated so many of the telia that further work was useless. In 1924 the telial material was of good quality.

An effort was made to test sporidia from all possible species of Ribes growing in that part of the United States. Varying amounts of material were collected from eight species—*Ribes americanum* Mill., *R. cynosbati* L.; *R. glandulosum* Grauer; *R. nigrum* L.; *R. odoratum* Wendl.; *R. rotundifolium* Michx.; *R. triste* Pallas; and *R. vulgare* Lam. The number of tests recorded in Table 3 is a fair index of the relative abundance of telia on the different species of Ribes in the eastern Adirondack region in 1923 and 1924.

About 350 series of experiments were made. Each series consisted of 2 to 12 slides bearing sporidia. In 1924 alone data were taken on more than 30,000 sporidia on the undried control slides, on more than 30,000 on the dried control slides, and on more than 64,000 which were exposed to various conditions. The data were based on representative samples of all the sporidia on each slide. The data were taken by moving the slide two or three times, both vertically and horizontally, across the microscope stage. The number of sporidia for 1923 was smaller than that for 1924. It is estimated that about 1,000,000 sporidia were present upon the slides in the two years, and probably this number was only a fraction of the total number produced by the teliospores, as many sporidia were lost in removing the telial columns from the slides.

METHODS

So far as possible sporidia from telia on newly collected Ribes leaves were used, but weather conditions at times made it necessary to use material which had been stored for three or four weeks. While storage of the telial material reduced the amount of telial germination, the sporidia produced by such material appeared to be as viable and vigorous as those from telia on newly collected leaves; that is, stored teliospores did not produce as many sporidia as fresh ones did, but the sporidia that were produced apparently were of maximum vigor.

GERMINATION OF THE TELIA

Various workers (*1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 18, 19, 27, 33, 36, 38, 39, 40, 41, 43, 51, 52, 54*) have investigated the morphology and, at least incidentally, the physiology of the germination of the teliospores of the Uredinales, but only a relatively few have included any Cronartiums in their investigations.

The oldest teliospores in a telial column of *Cronartium ribicola* are the terminal ones, as the length of the column is increased by the formation of new teliospores at its base (*18*). A newly formed telial column begins to germinate at its tip (*43*), and germination

proceeds downward as far as mature teliospores have been formed. Preliminary attempts were made to collect dry sporidia on slides by germinating the telia in situ on the Ribes leaves in moist chambers. After the proper germination period the leaves were supported over slides so that the mature sporidia could drop to the slides. Too few sporidia were obtained in this manner, so for the purpose of the tests reported in this paper the telial columns were germinated by the so-called "floating" method, which has been described in an earlier paper (*54*).

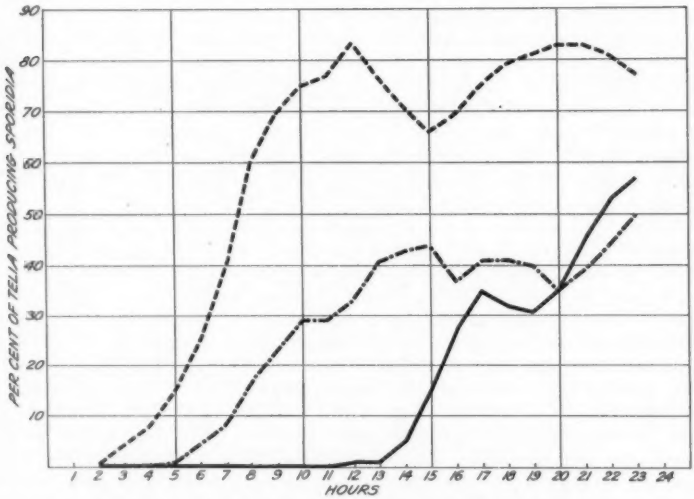

FIG. 1.—Time necessary in preliminary tests for the production of sporidia of *Cronartium ribicola*. The curves (3-hour moving averages) show the number of telial columns producing sporidia at any one time expressed in percentages of the number of columns examined at that time

Dash line: Sporidia produced from telia from *Ribes nigrum* stored 5 days indoors.
Dot-dash line: Sporidia producted from telia from *Ribes americanum* stored 15 days indoors.
Solid line: Sporidia produced from telia from *Ribes nigrum* stored 25 days indoors.

TIME NECESSARY FOR PRODUCTION OF SPORIDIA

Five preliminary tests with material stored for various lengths of time showed that the longer the period of storage the longer the time necessary for germination of teliospores and for production of sporidia. (Table 1 and fig. 1.) Also, the number of viable teliospores tended to decrease with increase in length of time in storage, dry (fig. 1). These facts were determined by hourly counts of germinated teliospores. Upon consideration of the above-mentioned tests, and of others in which hourly counts were not made, it was found that, for stored material, 18 hours might be taken as a standard germination period for obtaining an abundant supply of sporidia without excessive pregermination (germination before the preliminary drying of the sporidia). For fresh material a germination period of 12 hours was sufficient. Several attempts to shorten the germination period by but one hour resulted in scant sporidial production. Like increases in length of the germination period often resulted in increased pregermination. It is of interest to note that Robinson (*42*) found that

it took 12 hours to get a good crop of sporidia of *Puccinia malvacearum*. It is likely that temperature has much to do with the length of time needed for germination, as York and Snell (*63*), working at North Conway, N. H., found that only 5 to 6 hours were necessary for the germination of fresh teliospores and the production of sporidia of *Cronartium ribicola*.

TABLE 1.—*Number of hours necessary for the formation and germination of sporidia of Cronartium ribicola*

Ribes species	Collected		Storage period (days)	Hours from start of test to formation of first sporidia	Hours from start of test until number of telia producing sporidia reached the maximum	Hours from start of test to first germinating sporidia
	Place (N. Y.)	Date, 1923				
R. nigrum	Edinburg	Sept. 30	5	3	12	11
R. americanum	Warrensburg	Sept. 18	15	6	14	15
R. odoratum	Darrowsville	Sept. 12	20	6	13	17
R. nigrum	Edinburg	Sept. 7	25	13	23	20
R. nigrum	Wadhams	Aug. 23	29	8	24	20

PREPARATION OF SLIDES

Since it was impossible to collect a sufficient number of sporidia in a dry condition on glass slides, it was necessary to get them floating in water. After the teliospores had been allowed to germinate for the required time, the telial columns were floated on a clean dry slide. A drop of water was added to the telia on the slide, if it was necessary, and then the telia were stirred around briskly in the water to detach as many of the sporidia as possible from the promycelia. The telial columns were removed with a finely-pointed forceps. Next the fragments of columns were removed under a binocular microscope. The sporidia were then carefully examined for pregermination under a compound microscope. Usually there were few pregerminated sporidia on any slide, and their germ tubes were rarely more than 10 μ long. In all of the work with sporidia in the two years, only two or three secondary sporidia were found to have been formed as a result of pregermination; they were so rare that they were disregarded. In each group of experiments the amount of pregermination was quite uniform on all of the slides. Careful notes on the amount of pregermination were made for each slide. During the above operations the slides were not allowed to dry.

PRELIMINARY DRYING OF THE SPORIDIA

After the condition of the moist sporidia had been ascertained, they were dried. In the earlier tests this was done out of doors, and in the later ones it was done in a cellar where the atmospheric conditions were nearer the mean of the out door air conditions than those elsewhere. A small electric fan was used to hasten the drying of the slides in the cellar. The time required for drying the slides varied with the temperature and relative humidity, but it was seldom less than 5 and never more than 30 minutes. The time neceessary for drying, and the temperature and the relative humidity at which drying occurred, were recorded for every dried slide, except in the pre-

liminary work. As soon as a slide looked dry to the unaided eye it was examined for water under a compound microscope. If no water was detected the slides was considered to be dried.

EXPOSING THE DRIED SPORIDIA

Each slide of dried sporidia to be exposed was subjected to the desired atmospheric conditions, some out of doors, some in the cellar mentioned above, and some in chambers where these conditions were controlled. At the beginning and at the end of the exposure the temperature and relative humidity of the air were recorded. A recording hygrothermograph, which was checked once or twice daily by comparison with a thermometer and a sling psychrometer, gave data for the intervening time. At the end of the period of exposure each exposed slide was examined with a compound microscope for any water which might have condensed upon it. No condensation water was ever found on exposed slides, except in certain tests described below.

GERMINATION OF SPORIDIA

Each series of slides consisted of at least one exposed slide and one unexposed slide which served as a control. This control slide, called the "dried" control, was merely a slide which had received the same preliminary drying as the experimental one, but no subsequent exposure. The majority of the experiments included a second control slide, called the "undried" control, which remained wet throughout the experiment, receiving neither the preliminary drying nor any subsequent exposure. The undried controls showed the viability of the sporidia used in the experiments. The number of exposed slides in a series varied from 1 to 10, according to the factor being investigated. As soon as the telial columns had been removed from the undried control the control was placed in a small germination chamber.

As soon as the preliminary drying was finished the dried control was moistened with a thin film of clean, fresh, tap water. Usually this water film was applied with an atomizer, but in some experiments the dry slides were laid on a flat cake of ice where a very thin condensation film formed almost immediately on them. This method of wetting the dried slides is discussed more fully under the heading "Effect of icing on the viability of sporidia."

After the dried control had been wetted again, it was placed in the same germination chamber as the undried control of the same series. After being exposed and rewet the exposed slide was placed in the same germination chamber with its two controls. Where there were more than one exposed slide in a series, several germination chambers were necessary.

The germination chambers were kept in the above-mentioned cellar, during the germination period, because of the relatively uniform temperature there. Tests showed that no further germination took place in water cultures after about 24 hours. This agrees with Carleton's (*14*) general statement concerning the germination period of rust spores. It was also found that the germ tubes did not increase in length after about the same period of time. These facts made it possible to examine all of the slide of a series at the same time without introducing serious errors.

Massing of the sporidia sometimes resulted in no germination within the masses. This probably was an oxygen reaction, but it may have been a toxic one. Lack of germination was also noted when telial columns were badly massed (*54*), and it is apparently quite common when rust spores are massed (*24*).

EXAMINATION OF THE GERMINATED SPORIDIA FOR RESULTS

At the end of the germination period the sporidia were examined for germination under a compound microscope. Records were made of the number of sporidia counted, the number germinating, the length of germ tubes formed, and the presence of secondary sporidia. The primary and secondary sporidia were of the same approximate

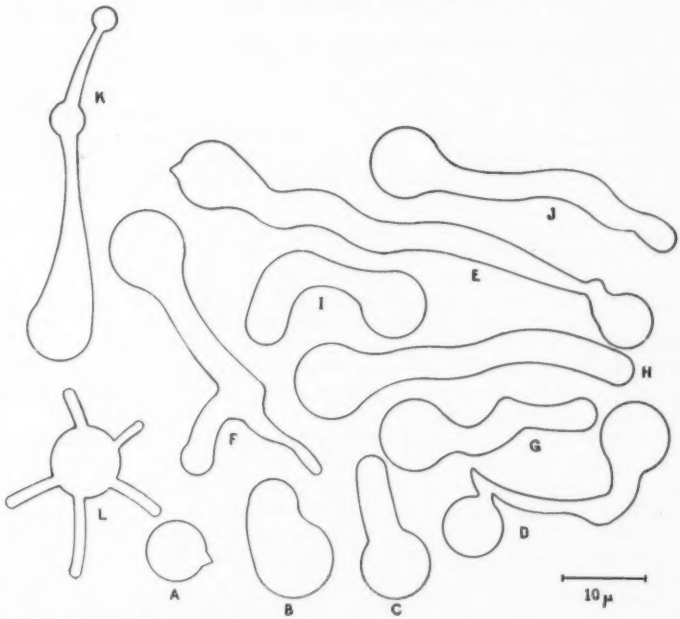

FIG. 2.—Germination of sporidia of *Cronartium ribicola*: A, ungerminated sporidium; B, C, Slight germination of the type referred to as "pregermination" in this paper; D, E, Secondary sporidia; F, branched germ tube; G, H, I, J, vigorous germination; K, tertiary sporidium produced by a secondary sporidium; L, sporidium with several abnormally slender and rather short germ tubes

size, and they resembled each other so closely in every way that the writers could not distinguish one from the other. The presence of secondary sporidia in large numbers often decreased the apparent germination percentages, because many of them were detached in placing the cover glasses before examination. Since these secondary sporidia had not begun to germinate, they increased the number of ungerminated sporidia counted. Germ tubes of primary sporidia which have produced secondary sporidia are likely to be sharply pointed (*16*, *18*). In some slides the number of these pointed germ tubes nearly equalled the number of ungerminated sporidia found. Figure 2 shows some of the various forms of germ tubes produced.

The germ tubes produced by the sporidia in the tests ranged in length from 10 to 100 μ. Germ tubes 50 to 60 μ in length were not uncommon. Vigorous germination yielded germ tubes 25 μ or more in length. The lengths of the germ tubes were important, because they made it possible to distinguish between growth which had occurred before the sporidia were subjected to the preliminary drying and growth which occurred after that time. The presence of secondary sporidia was also important in this connection, because they were practically never found in situ as a result of pregermination. Their presence in numbers subsequent to this stage of the experiments could be due only to their formation after the preliminary drying and the exposure of the sporidia.

CORRELATING THE DATA

There was little time in the field laboratory to correlate the results, as all efforts were bent upon clearing up certain practical questions concerning the behavior of these erratic, and, in some respects, very easily affected spores. When the data were analyzed it was found that many gaps still existed. No excuse is offered for this, because all possible data were taken. Since further work upon this problem is deemed inadvisable at present, it is believed that the data should be published so as to be available to future investigators. The results are presented largely by means of graphs, because of the great condensation which is possible by this method of presentation. Publication of detailed tables would be out of the question.

While preparing the data, it was found that although sporidia were very resistant to some adverse conditions, they were at times very sensitive to minor changes in environment. For this reason, the results from a number of early experiments, in which all of the sporidia on both the control and exposed slides had succumbed to some obscure and undetected condition, were discarded.

As stated earlier, the air temperature and relative humidity were recorded for each exposed slide in every experiment. However, the same relative humidity at different temperatures may result from different actual amounts of atmospheric moisture. Saturation deficit, which can be computed from temperature and relative humidity, is an index of the drying power of the air. The writers' data of air temperatures and relative humidities have been converted into saturation deficits in millimeters, in order to reduce these two different variables to a common basis. Saturation deficit can be computed easily by means of meterological tables (*45*) and a formula given by Hann (*29*). Or, relative humidity and temperature can be converted directly into saturation deficit by one reading from a graph published by Meyer (*37*). Both methods gave the same results.

After the saturation deficits had been calculated, the data were arranged in groups or classes according to the saturation deficit of the air to which the sporidia were exposed. Usually the air conditions remained within one saturation-deficit class during the whole exposure period for each slide. In those cases where the saturation deficit varied from one class to another, the data were grouped in the class of the greatest saturation deficit occurring during the exposure.

Throughout this paper the term "weighted three-point moving average" is used to indicate a moving average each point of which

is weighted by the number of tests made at that point and by the number of tests made at the points on either side. For example, suppose 10 tests with an average of 70 per cent were made at a saturation deficit of 2, 5 tests with an average of 80 per cent at a saturation deficit of 3, and 20 tests with an average of 60 per cent at a saturation deficit of 4. The value for saturation deficit 3 in the weighted three-point moving average would be

$$\frac{(10\times 70 \text{ per cent})+(5\times 80 \text{ per cent})+(20\times 60 \text{ per cent})}{10+5+20}=66 \text{ per cent.}$$

The value of each point in the moving average was determined in this way.

EFFECT OF DRYING ON THE VIABILITY OF SPORIDIA

Unless it germinates immediately after maturing, practically every sporidium produced in the fields and forests of this country is subjected at some time to some degree of desiccation. Buller (*12*) and others (*9*, *21*, *34*, *36*) found that the sporidia of many of the Uredinales are thrown forcibly from the sterigmata. Buller also observed that at the time of maturity each sporidium of some genera of the rusts has at its base a tiny droplet of water and that when the sporidium is thrown off the water drop goes with it. This is a most remarkable provision against desiccation. Unfortunately no species of Cronartium has yet been carefully examined to determine whether it is like other genera of the Uredinales in these respects. It is likely that it is, however. Since the susceptibility of sporidia to desiccation may be decisive in controlling the amount of infection which they may cause in white pines, the effect of drying on their viability is very important from a practical standpoint. This problem was one of the most urgent of all those connected with the physical factors controlling the functions of the sporidia of *Cronartium ribicola*. It was attacked from several different angles.

Throughout the present investigation the sporidia were studied on glass slides. Heald and Studhalter (*31*), working with the spores of *Endothia parasitica* (Murrill) A. and A., found "that conditions of desiccation on a glass surface are more severe than on surfaces on which spores would be dried under natural conditions in the field." They concluded that tests made from spores on glass surfaces did not give the maximum longevity of either ascospores or pycnospores of *E. parasitica*. In view of these results there is a possibility that sporidia of *Cronartium ribicola*, if they had been dried on some other surface besides glass, would have been even more tolerant of drying than they appeared in any of the reported tests. No comparative tests were made to determine this point, because time was limited. Some longevity tests of sporidia dried on epidermis of Ribes leaves or of pine needles and on pine bark are very desirable.

EFFECT OF THE PRELIMINARY DRYING ON THE VIABILITY OF THE SPORIDIA

Since large numbers of sporidia could be obtained only in a wet condition, it was imperative to determine the effect of the preliminary drying to which all sporidia to be exposed were subjected before any experiment could be started. For this reason every experiment consisted of a series of slides of sporidia. Every series contained

an exposed slide or slides, and a dried control slide; and the majority of them also included an undried control slide which represented the vigor of the sporidia before any drying took place. The effect of the preliminary drying has been considered from various angles.

In Figure 3, the percentage of germination of the sporidia on control slides dried at saturation deficits up to 5 mm. are compared with those of the directly comparable undried controls. Inspection of the graph indicates that, on the whole, the undried sporidia germinated better than the dried ones.

The average of the percentages of germinations for the undried control slides was 61.1, while that for the strictly comparable dried controls was 42.7, a difference of 18.4 per cent in favor of the undried ones. When all the experimental data (including those from slides which were not directly comparable) were utilized, the results were practically the same as when only strictly comparable data were used.

The weighted averages of the results in the strictly comparable experiments were obtained as follows: In the undried controls 25,743 sporidia were counted, of which 15,999, or 62.1 per cent, germinated;

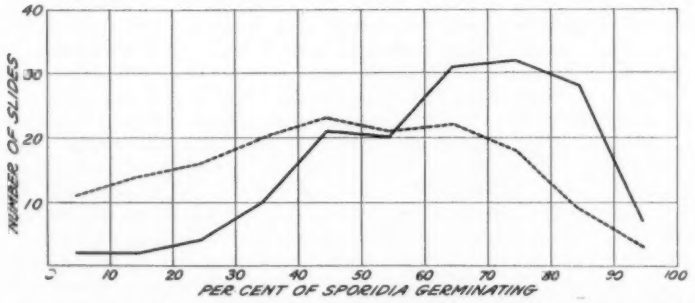

FIG. 3.—Distribution of the germination percentages of sporidia of *Cronartium ribicola* in the controls dried at saturation deficits up to 5 mm., and the distribution in the directly comparable undried controls
Solid line: Undried controls.
Dash line: Dried controls.

in the strictly comparable dried controls, 26,027 sporidia were counted, of which 11,530, or 44.3 per cent, germinated. The difference between the two was 17.8 per cent. When all of the experimental data (including those from slides which were not strictly comparable) were used, the results were practically the same.

Both of these methods of averaging indicate that the preliminary drying reduced the average germination by approximately 18 per cent, on the basis of 100 per cent possible germination. On the basis of the germination actually occurring in the cultures, it reduced germination about 29 per cent.

While the difference between the averages for the dried and undried sporidia is large, the high variability shown in Figure 3 makes desirable some further examination of the significance of this difference. In the pairs of directly comparable slides the undried slides not infrequently gave lower percentages of germination than did the dried ones. However, it is evident from Table 2 that in every one of the 14 groups into which the tests were divided, the percentage of germination on the undried slide was greater than that on the dried

one in more than half of the pairs of slides. Figure 4 gives further information on the relative constancy of the superiority of the undried slides. The results are so consistent as to leave no doubt concerning the general harmful effect of the preliminary drying upon the viability of the sporidia.

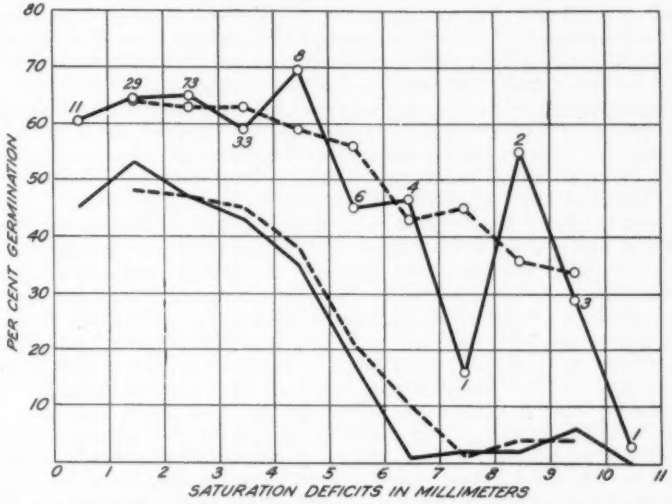

FIG. 4.—Comparison of the germination of sporidia of *Cronartium ribicola* in the controls dried at various saturation deficits with that in the strictly comparable undried controls. The figure at each point represents the number of experiments upon which each point is based. This number is the same for both the undried and the dried controls

Solid line: Germination in the dried controls.
Dash line: Weighted 3-point moving average of the same results.
Circle-solid line: Germination in the undried controls.
Circle-dash line: Weighted 3-point moving average of the same results.

TABLE 2.—*Relation between the injury caused by the preliminary drying of the sporidia of Cronartium ribicola and the saturation deficits at which the drying was done*

Ribes hosts of telia from which sporidia came	Number of comparable pairs of slides in which the dried one was subjected to a saturation deficit of—				Percentage of comparable pairs of slides in which undried ones germinated better than the dried ones when the latter were subjected to a saturation deficit of—			
	0–2.9 mm.	3–4.9 mm.	5–6.9 mm.	7–10.0 mm.	0–2.9 mm.	3–4.9 mm.	5–6.9 mm.	7–10.0 mm.
R. nigrum	83	23	8	7	68	78	100	100
Other species besides R. nigrum (the five following)	30	18	2	.....	77	89	100	.....
R. cynosbati	5	.....	.....	.....	80	.....	.....	.....
R. glandulosum	13	4	1	.....	69	100	100	.....
R. odoratum	.....	5	.....	.....	.....	80	.....	.....
R. rotundifolium	10	7	1	.....	80	86	100	.....
R. triste	2	2	.....	.....	100	100	.....	.....

In Table 2 the difference in favor of the undried sporidia was more consistent at the higher saturation deficits than at the lower ones. Figure 4 shows clearly that the magnitude of the difference in percentages of germination in favor of the undried sporidia increased steadily with increase of the saturation deficit. Because the number of experiments at some of the greater saturation deficits was small, the moving average shown by the broken line is the best basis for comparison. The material used in the experiments at the greater saturation deficits happened to be poorer, on the whole, than that used at the smaller saturation deficits. As a consequence of the cramping of percentages near the end of the scale, it is reasonably certain that the differences between dried and undried sporidia in the experiments at the greater saturation deficits would have been even larger than it was if the material had been as good as that used in the experiments at smaller saturation deficits.

Comparison of the results with sporidia from different species of Ribes in Table 2 makes it appear that the preliminary drying caused less damage to sporidia from the very congenial host *Ribes nigrum* than to sporidia from less congenial hosts. Comparison of the results from sporidia produced on *R. nigrum* with the results from sporidia from all of the other species of Ribes (given in the next line below *R. nigrum* in Table 2) shows that in both of the lower saturation-deficit groups the difference in favor of the undried sporidia was about 10 per cent greater for other species than for *R. nigrum*. In each column, however, the difference between the percentages for sporidia from the two sources was no greater than the standard error, and the combined differences obtained from the two columns is less than one and one-half times its standard error, so the difference is of doubtful significance. There is about 1 chance in 16 that the difference in the effect of the drying was accidental. The comparison presented in Table 2 gives no information on the magnitude of the difference between the effect of the drying on sporidia from *R. nigrum* and its effect on those from other Ribes species. The average of the germination percentages of sporidia from *R. nigrum* which received their preliminary dryings at saturation deficits between 0 and 6.9 mm. was 48 per cent; that of the comparable undried controls was 66 per cent. The preliminary drying reduced the average germination by approximately 18 per cent, on the basis of 100 per cent possible germination. On the basis of the germination actually occurring in the cultures it reduced germination about 27 per cent. The average of the germination percentages of sporidia from other species of Ribes which received their preliminary dryings at saturation deficits between 0 and 6.9 mm. was 35 per cent. That of the comparable undried controls was 59 per cent. The preliminary drying reduced germination about 24 per cent, on the basis of 100 per cent possible germination. On the basis of the germination actually occurring in the cultures it reduced germination about 41 per cent. The averages weighted by the number of sporidia counted and by the number germinating on each slide were approximately the same as those obtained by merely averaging the germination percentages of each slide.

Since a sporidium which does not germinate can not infect a white pine needle, even if all other conditions are favorable, the fact that even a brief drying reduces germination means that it reduces the possible number of infections from sporidia.

EFFECT OF ALTERNATE WETTING AND DRYING ON THE VIABILITY OF SPORIDIA

The effect of alternate wetting and drying of sporidia on their viability and their power to infect pines is another question of considerable practical importance, because in nature the sporidia are subjected to such conditions when dew or showers alternate with sunshine. Four groups of experiments yielding data on this problem were conducted with sporidia from different collections of *Ribes nigrum*. These included 14 series of tests, in 11 of which the films of water germinating the sporidia were obtained by laying the slides on ice for 1 or 2 minutes. In the first group of experiments some of the slides were dried and then wet once, some twice, and some three times. In the second and third groups the test slides received 1 to 5, and in the fourth group 1 to 11, alternate wettings and dryings.

Figure 5 gives a comparison of the germination of sporidia on iced slides which were dried 1 to 5 times at saturation deficits of 5 to 9 mm., with the germination of sporidia on iced slides dried at saturation deficits below 4 mm. The former were injured more by the drying than by the latter, beginning with the first drying and continuing to the fifth. The saturation deficits above 5 mm. decreased germination from the beginning, while those below 4 mm. did not begin to decrease germination until the third drying. In other words, the influence of the saturation deficits above 5 mm. was more marked than that of those below 4 mm.

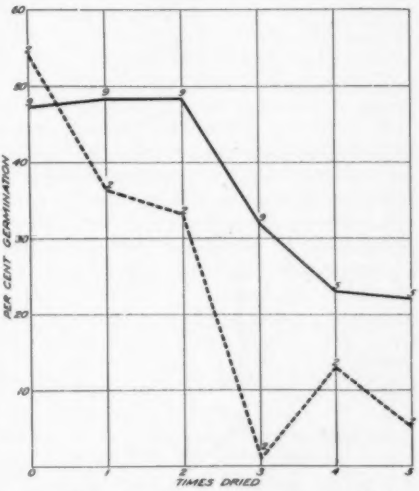

FIG. 5.—Effect of one to five alternate wettings and dryings on the germination of sporidia of *Cronartium ribicola*. All sporidia were wet by laying the slides on ice for 1 or 2 minutes. The figure at each point is the number of tests on which the point was based

Solid line: Germination of sporidia dried at saturation deficits of 2 to 4 mm.

Dash line: Germination of sporidia dried at saturation deficits of 5 to 9 mm.

Figure 6 gives a comparison of the comparable iced and uniced sporidia which were dried at saturation deficits of less than 4 mm. The trend of both graphs is downward, but the one for the iced sporidia does not drop as low as does that for the uniced ones until the eighth drying. Beginning with the sixth drying, there is but one experiment for each of the following points. Hence the data for these should receive less weight than those based upon more experiments.

Even 11 alternate wettings and dryings did not kill all of the sporidia. This fact is emphasized more by the length of the germ tubes produced than by the percentages of germination. The maximum lengths of germ tubes produced in the two series in which there were 1 to 11 alternate wettings and dryings are shown in table on page 414.

When these same slides were examined for evidences of pregermination it was found that pregermination was absent or slight, and that the tubes ranged from approximately 5 to 15 microns in length.

Number of times dried	Maximum lengths of germ tubes produced by—	
	Iced sporidia	Uniced sporidia
	Microns	*Microns*
0	40	60
1	50	40
2	50	50
3	50	20
4	25	30
5	30	50
6	30	30
7	60	
8	20	50
9	20	30
10	20	40
11	30	20

It is clearly shown by the above results that while a single brief period of dryness is harmful, as is shown by a comparison of the results obtained with the undried and the sporidia dried once (page 409), repeated drying is not as deadly as one would expect. In fact, a few sporidia showed remarkable resistance and germinated strongly, even after 11 dryings. Alternate wetting and drying was, on the

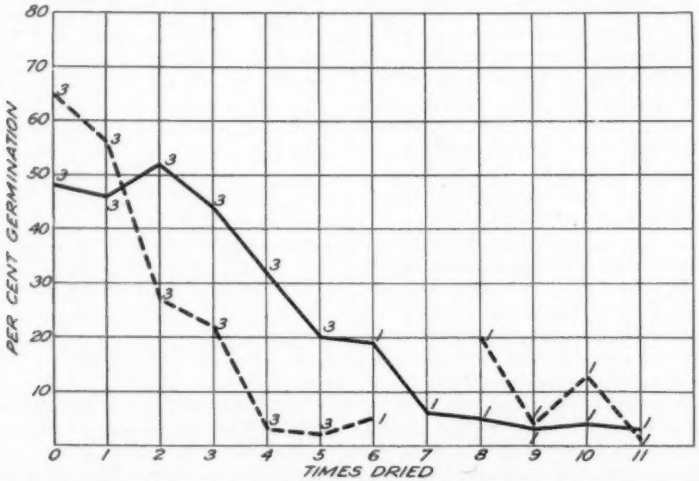

FIG. 6.—Comparison of the germination of iced and uniced sporidia of *Cronartium ribicola* alternately wet and dried 1 to 11 times at saturation deficits of 2 to 4 mm. The figure at each point is the number of tests on which the point was based

Solid line: Germination of iced sporidia.
Dash line: Germination of uniced sporidia.

whole, less injurious when the sporidia were wet by icing than when they were wet by spraying. Drying of the iced sporidia was somewhat quicker than that of the uniced ones, as the film of water was thinner. It may be possible that if the sporidia had remained dry longer between wettings more injury would have been noted.

EFFECT OF PROLONGED DRYING UNDER UNCONTROLLED CONDITIONS ON THE VIABILITY OF SPORIDIA

To get an idea of the possible longevity of sporidia under the conditions existing out of doors during the season in which infection of pines occurs, slides of sporidia which had received the preliminary dryings were exposed to uncontrolled atmospheric conditions. These conditions were liable to sudden and decided change, as the weather at the season and place where the work was done was capricious. The relative humidity and temperature varied more or less while the experiments were in progress. At the beginning and at the end of each test the relative humidity and the temperature were recorded. From these the saturation deficits were calculated and the data were grouped into saturation-deficit classes. Where the conditions varied between two classes, the data was placed in the greater saturation-deficit class. Figures 7, 8, and 9 show the percentages of germination for the controls which had received only the preliminary dryings, and those for the sporidia which were further dried during exposure. They show that exposure of sporidia at all saturation deficits had an unfavorable effect on viability, but that some sporidia survived at all the saturation deficits in the tests.

In Figure 10 is a graph for each of the saturation-deficit classes, based on the difference between the average germination on the dried control slides and that on the exposed slides. Because of fragmentary data for the larger saturation deficits, the isolated points were connected with broken lines to show the general trend of each graph. Up to 12 hours' exposure, the graphs for the three classes run as would be expected—i. e., in the class of the smallest saturation deficit there was less difference between the dried controls and the exposed sporidia than there was in the other two classes. The graph for the medium saturation-deficit class occupies the middle position, and that for the class of greatest saturation deficit gives the greatest differences. This general trend is apparent throughout the graphs, in spite of the erratic fluctuations occurring at the longer times of exposure, these fluctuations probably being due largely to the scantiness of data. In general, within each saturation-deficit class the difference between the average germination of the exposed sporidia and that of the dried controls increased with the length of the exposure.

Table 3 gives data of the tests on the longevity of sporidia under natural (uncontrolled) conditions for the various species of Ribes from which the telia were taken. Three saturation-deficit classes, representing temperatures ranging from 32–86° F. and relative humidities ranging from 58 to 100 per cent, are used to show the conditions under which the sporidia were exposed. The upper limit of the third class was not the same for all species. Because the conditions were not controlled, the distribution of these tests in the three classes is purely accidental. The third column in Table 3 shows the tests in which germination occurred after the length of exposure indicated. The amount of germination is not given. The last column gives similar data for those tests in which no germination occurred after exposure. Figures 7, 8, and 9 show the amount of germination which occurred in these tests. Attention is called to the fact that there were relatively few tests in which germination entirely failed, even under severe conditions. It is believed that the failure to

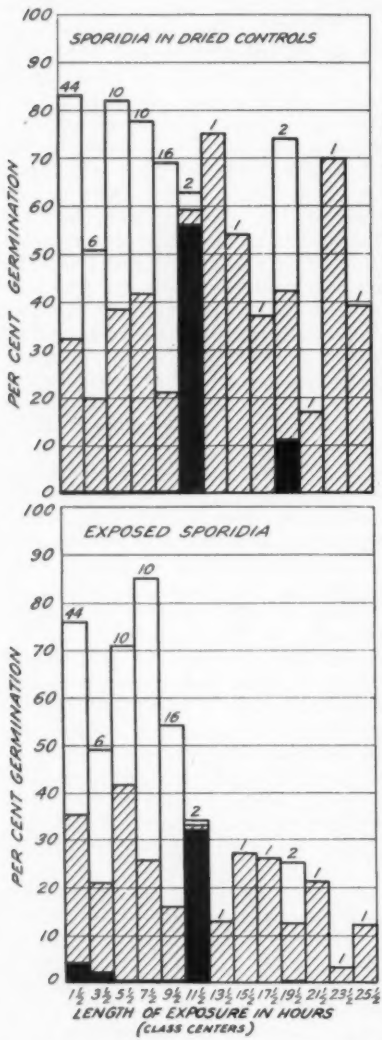

FIG. 7.—Germination of sporidia of *Cronartium ribicola* exposed to saturation deficits between 0 and 2.9 mm. The control slides had received the same preliminary drying and were comparable with the exposed ones, except for the exposure. The figure above each bar is the number of tests

Black portion of bar, germination of the poorest slide; shaded portion, germination of the average slide; white portion, germination of the best slide.

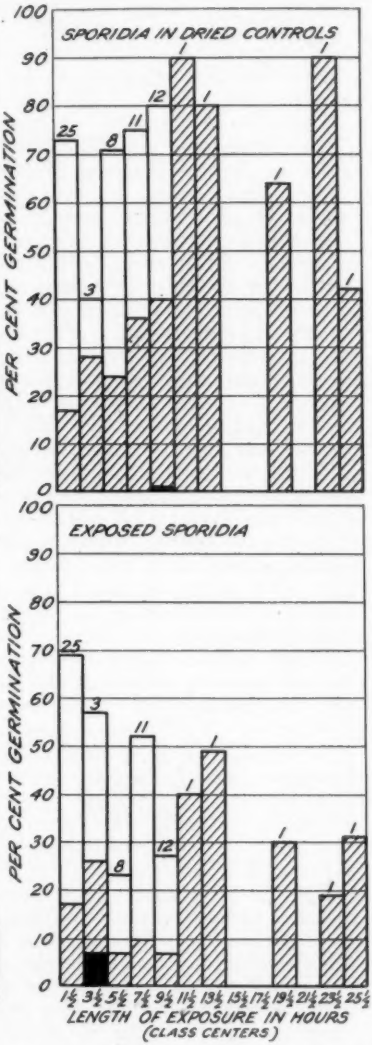

FIG. 8.—Germination of sporidia of *Cronartium ribicola* exposed to saturation deficits between 3 and 4.9 mm. The control slides had received the same preliminary drying and were comparable with the exposed ones, except for the exposure. The figure above each bar is the number of tests

Black portion of bar, germination of the poorest slide; shaded portion, germination of the average slide; white portion, germination of the best slide.

germinate in certain of the tests is not due to the saturation deficits of the exposures but to undetected causes. Germination occurred in some of the tests at all periods of exposure up to 26 hours in length. No attempt was made to expose slides long enough to kill all of the sporidia, because the knowledge that they can remain viable in an air-dried condition for 26 hours is sufficient for all practical purposes. The vigor of germination of the sporidia exposed longest is indicated not only by the percentage of germinating sporidia but also by the maximum lengths of germ tubes formed in the cultures. The maximum tube lengths for the longest exposed sporidia from *Ribes rotundifolium* and *R. triste* were approximately 20 μ, only about 10 μ less than the maximum of those produced by the undried sporidia in the same series; and those for *R. odoratum* and *R. vulgare* were approximately 30 μ. No undried sporidia were tested in the same series. The maximum tube lengths for the longest exposed sporidia from *R. cynosbati* and *R. glandulosum* were approximately 40 μ, which equaled the maximum for the undried sporidia in the same series. The maximum tube lengths for sporidia from *R. nigrum* exposed 26 hours was 30 μ, which equaled the maximum for the unexposed sporidia in the same series. These figures seem to indicate that the prolonged drying did not reduce the vigor of the surviving sporidia as much as it did the percentage of germination.

Although the point has not been investigated for dried sporidia, it has been assumed that a sporidium which is capable of germinating is also capable of infecting white pines, if other conditions are suitable for infection to take place. In order to estimate the possible maximum number of viable sporidia per unit of leaf surface, the maximum germination percentages obtained after the longest exposures for

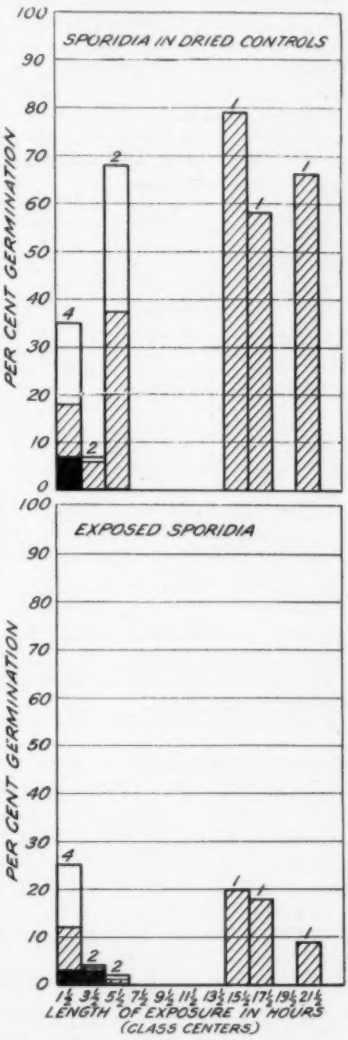
SPORIDIA IN DRIED CONTROLS
PER CENT GERMINATION
EXPOSED SPORIDIA
PER CENT GERMINATION
1½ 3½ 5½ 7½ 9½ 11½ 13½ 15½ 17½ 19½ 21½
LENGTH OF EXPOSURE IN HOURS
(CLASS CENTERS)

FIG. 9.—Germination of sporidia of *Cronartium ribicola* exposed to saturation deficits between 5 and 9.5 mm. The control slides had received the same preliminary drying and were comparable with the exposed ones, except for the exposure. The figure above each bar is the number of tests

Black portion of bar, germination of the poorest slide; shaded portion, germination of the average slide; white portion, germination of the best slide.

each Ribes species were applied to the potential maximum sporidial production estimates given by Taylor (*56*). These give the astonishing figures in the fifth column of Table 4. Each of these sporidia would be potentially capable of causing infection of white pines, but as a matter of fact only a very small fraction of them would ever reach pine needles and very few of those that reach pine needles ever cause infection. Observational data show that this is the case. Further longevity tests are needed to get an average figure for the third column of Table 4. The writers in a previous paper (*53*) have discussed some of the conditions which would presumably result in white pine infections.

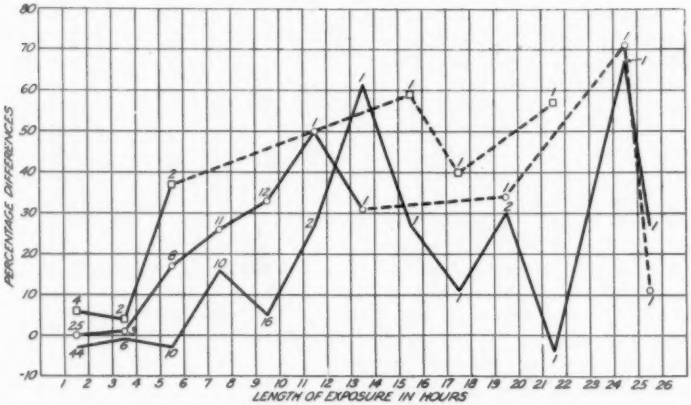

FIG. 10.—Differences between the average germination percentages of the exposed sporidia of *Cronartium ribicola* and those of the controls which had received only the preliminary drying. The figure at each point is the number of experiments upon which the point is based

Solid line: Exposures to saturation deficits between 0 and 2.9 mm.
Circle, solid line: Exposures to saturation deficits between 3 and 4.9 mm.
Square, solid line: Exposures to saturation deficits between 5 and 9.5 mm.
The broken sections of the lines indicate that the data were fragmentary.

TABLE 3.—*Longevity of sporidia of Cronartium ribicola from various Ribes hosts*

Ribes species	Saturation deficits (mm.)	Hour of exposure followed by germination	Hours of exposure followed by failure to germinate
R. americanum	0-2.9	½; 1; 2; 3; 4; 5; 9½	
R. cynosbati	0-2.9	5; 6; 7; 7; 8; 8; 9; 9; 10; 10	9
	3-4.9	2	6
	5.0-5.7	1	
R. glandulosum	0-2.9	1; 1½; 2; 3; 4; 4; 5; 6; 7; 8; 9; 10	
	3-4.9	½; ⅔; ⅚; 1; 1; 1¼; 2; 2	
	5.0-9.5	3; 4; 6	5
R. nigrum	0-2.9	½; ½; ½; ½; ½; ½; ½; ½; ½; ⅔; ⅚; ⅚; 1; 1; 1¼; 1¼; 1¼; 1½; 1½; 2; 2; 4; 5; 6; 6; 7; 8; 8; 9; 10; 10; 12; 14; 16; 18; 20; 22; 24; 26.	5; 7; 9; 20½
	3-4.9	½; ½; ½; ½; ½; ½; ½; ⅔; ⅚; 1; 1¼; 2; 6; 8; 8; 9; 9; 10; 12; 14; 20; 24; 26.	1; 1; 8; 10
	5.0-5.7	½; 16; 18; 22	
R. odoratum	0-2.9	½; ½; ⅔; ⅔; ⅚; ⅚; 1; 1; 1½; 1½; 2; 2; 10	
	3-4.9	6; 7; 8; 9; 10	
R. rotundifolium	0-2.9	⅚; 1; 1¼; 1½; 2; 6; 8½; 9; 11½	
	3-4.9	⅔; 2; 3; 4; 4; 5; 5; 6; 6; 7; 7; 9; 9; 10; 10	8; 8
	5.0-6.2	½; 2	
R. triste	3-4.9	8; 9	6; 7; 10
R. vulgare	0-2.9	9	

TABLE 4.—*Potential number of viable sporidia of Cronartium ribicola per square inch of leaf surface after prolonged exposures while dry*

Ribes species	Hours of exposure	Maximum percentage of sporidia germinating after the exposures given in the second column	Potential production of sporidia per square inch [a]	Number of potentially viable sporidia per square inch after the exposures given in the second column
R. triste	9	12	360,000	43,200
R. americanum	9½	54	974,280	526,111
R. cynosbati	10	31	1,141,920	353,995
R. vulgare	9	11	1,305,000	143,550
R. glandulosum	10	22	1,679,484	369,486
R. rotundifolium	11½	32	2,746,140	878,765
R. odoratum	10	11	5,480,640	602,870
R. nigrum	14	49	16,799,400	8,231,706
R. nigrum	26	31	16,799,400	5,207,814

[a] From publication by Taylor (*56*).

EFFECT OF PROLONGED DRYING UNDER CONTROLLED CONDITIONS ON VIABILITY OF SPORIDIA

As a phase of the investigations of the effect of the relative humidity and temperature of the surrounding air upon sporidia exposed dry, experiments were performed in which the relative humidity was controlled. This was done by means of sulphuric acid, a method long known and used by chemists for removing the water vapor from air contained in small chambers. So far as known to the writers, this method was applied only rather recently to controlling relative humidity of air in which fungus cultures were grown (*30*, *55*, *57*, *65*). Scheibler desiccators of approximately 5,000 c. c. total capacity were used. Four hundred c. c. of sulphuric acid of known density was put in the base of each desiccator. This amount of acid was used because it presented a maximum surface area for the most rapid action on the air, and with such a volume the density of the acid would be changed very little by the water taken up from the air. Acid known in the trade as C. P., and of a specific gravity of 1.84, was used in making up the solutions. The acid was diluted with crushed ice in a flask embedded in ice and water. Density was ascertained with a hydrometer. Curtis (*20*) gives a graph showing the relative humidity which each density of sulphuric acid maintained in an inclosure. This graph appeared to be the most complete source for such data, his data agreeing within experimental error with data from other sources (*55*), and it was used in preparing the acid solutions for these experiments. As soon as the acid solution was put in the chamber, the cover was sealed with vaseline and the chamber was placed in the above-mentioned cellar, where the temperature was quite uniform. The acid was put in the chambers at least 12 hours before the beginning of a test, so that the relative humidity of the air could reach equilibrium. Tests were run simultaneously in five chambers, each at a different relative humidity. The covers were fitted with ground glass stoppers, the openings for which were large enough for an ordinary glass microscope slide to pass through them easily. A slide bearing sporidia could be inserted or taken out through these openings in about 10 seconds. This

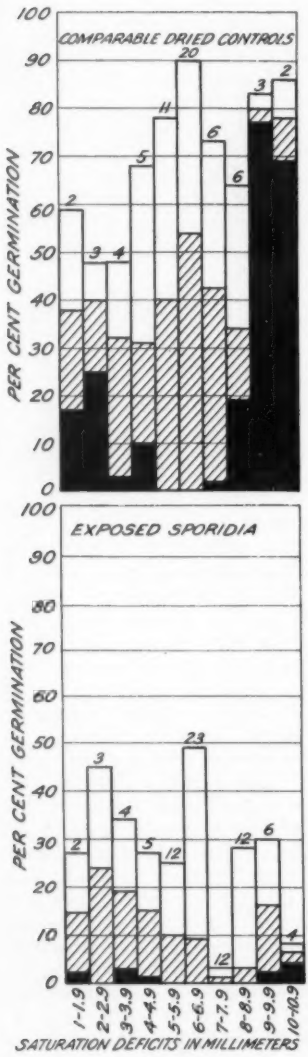

FIG. 11.—The germination of sporidia of *Cronartium ribicola* in the desiccators 3½ hours. The control slides received only the preliminary drying. The figure above each bar is the number of tests. In a few cases there were more exposed slides than controls, because a single control slide sometimes served for two exposed ones

Black portion of bar, germination of the poorest slide; shaded portion, germination of the average slide; white portion, germination of the best slide.

brief period allowed but little interchange of air, especially as the air surrounding the chambers was of the same temperature as that within them. Liberal time was allowed for the experiments, so that the relative humidity of the air could become stabilized after the opening of the chamber. A few experiments ran for 1½ hours, and a few others for 5½ hours, but most of them ran 3½ hours. In the chambers, the slides of sporidia rested in a horizontal position upon a loose glass plate supported by a perforated porcelain plate. The slides were about half way, vertically, between the cover of the chamber and the surface of the acid. After a series of experiments was completed, the density of the acid was again determined. The temperature in the chambers was read without opening them, directly from thermometers lying on the glass plates beside the slides. Fluctuations of the temperature outside the chambers were so slight that there was practically no variation in the chambers during an experiment.

Eleven experiments were run at various times, with different material, and with saturation deficits ranging from 1.0 to 10.9 mm. which represented temperatures ranging from 53° to 70° F. and relative humidities ranging from 41 to 86 per cent. Most of the sporidia were wet by spraying, but a few were wet with the condensation water resulting from placing the slides on ice. Figure 11 shows (much condensed) the results of all of the 3½-hour tests. Since all factors, except that of saturation deficit at which they were exposed, are constant throughout each test, no error results from combining the results of both iced and uniced slides in the same graph. Figure 11 shows clearly that the material used for the tests at the higher saturation deficits happened to be most vigorous originally. This was purely accidental. Figure 12 represents the ratio of the average germination of the exposed sporidia to that of their dried controls. It shows that the smaller saturation deficits gave the higher per-

centages of germination, and that there was a decrease of germination as the saturation deficit increased. Figure 13 shows the differences obtained by subtracting the average percentages of germination of the exposed sporidia from those of their dried controls. The two methods of comparison give results that are in essential agreement with each other. Both show clearly that the injury to the sporidia from exposure increased with the increase of saturation deficit at which they were exposed.

Figure 14 shows the data for all comparable tests run at the three periods of exposure—1½, 3½ and 5½ hours—grouped according to the saturation deficits at which they were run, viz, 2.0 to 2.9 mm.

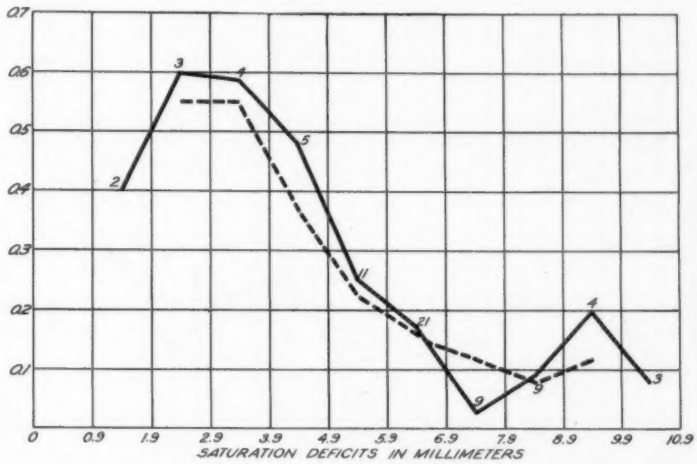

FIG. 12.—The ratio of the average germination percentages of sporidia of *Cronartium ribicola* in desiccators, 3½ hours to the average germination percentages in the comparable controls. The figure at each point represents the weight given that point in making the 3-point moving average. This weight was obtained by averaging the number of control slides and the number of exposed slides, halves being dropped in all cases

Solid line: Ratio between the germination percentages in exposed and the control slides.
Dash line: Weighted 3-point moving average of the same data.

3.0 to 4.9 mm., and 5.0 to 6.9 mm. The available data are limited, but they show quite clearly a general trend of higher germination at the smaller saturation defecits, and an earlier falling off of germination at the greatest saturation deficit. Also, increase in length of time of exposure tends to decrease germination. The graph for the saturation deficit class 3.0 to 4.9 mm. is abnormally irregular, but even with it the tendency is downward with increase in time of exposure.

EFFECT OF SUNSHINE ON VIABILITY OF SPORIDIA

In 1923 nine series of preliminary tests on the effect of direct sunshine on the viability of sporidia were run. In these tests a recording hygrothermograph was set in the full sunshine. The sporidia were exposed upon the black, perforated, metal plate, located just over the

hairs of the instrument and scarcely an inch from them. The material used was of low viability, as was all of that used in 1923. However, the controls, which had had the usual preliminary drying, gave consistently higher germination than did the sporidia which had been exposed to the sun for 2 to 12 minutes. Low percentages of germination were obtained after such exposure with the saturation deficit ranging from 1.0 to 18.0 mm. Sporidia which germinated after exposure to sun at a saturation deficit of 8.5 mm. produced germ tubes 20 μ in length, and secondary sporidia. No undried sporidia

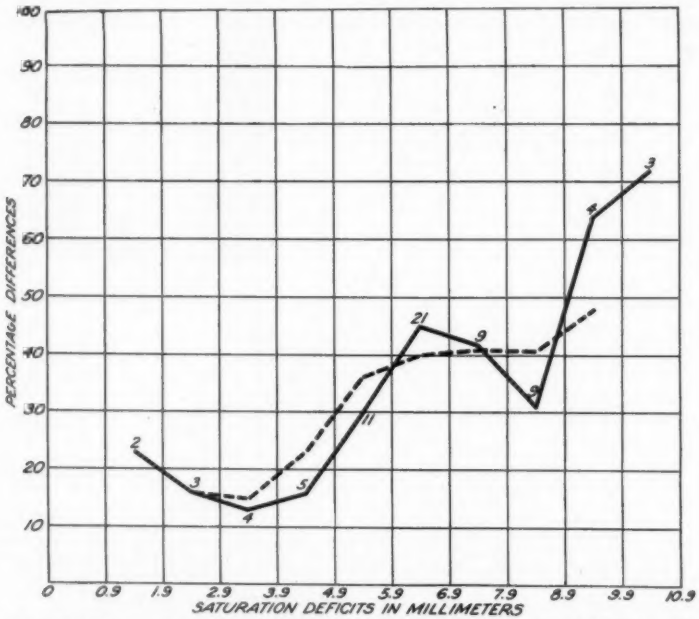

FIG. 13.—The differences between the average germination percentages of sporidia of *Cronartium ribicola* in desiccators 3½ hours and those of the controls which had received only the preliminary drying. The figure at each point is the weight given that point in making the 3-point moving average. This weight was obtained by averaging the number of control slides and the number of exposed slides, halves being dropped in all cases

Solid line: The differences between the germination percentages in the exposed and the control slides.
Dash line: Weighted 3-point moving average of the same data.

were tested in these series, so it is impossible to state how long the germ tubes would have been which these sporidia would have produced prior to their preliminary drying and exposure.

In 1924 an attempt was made to eliminate the heat of sunlight as an injurious factor. In the first attempt it was expected that the heat from the sun would be great enough to prevent water vapor from condensing on the slides. The sporidia were exposed to sunlight within Petri dishes lined below with moist filter paper, and set upon a flat ice surface. A film of water condensed immediately on the upper surfaces of the slides, thus wetting the sporidia. For the six series of tests thus made, the average of the percentages of germination in the

dried controls and that of the sporidia exposed as above decribed was practically equal, being 31 and 32 per cent respectively. That is, the water film, the glass cover, or the low temperature, prevented any injury from the exposure to the sun. Sporidia exposed at the same time by placing the slides on a light-colored surface in direct sunlight were decidedly injured. This may mean that the heat rays rather than the visible rays of sunlight injure sporidia, or that ultra-violet rays or some other rays which glass cuts out injure them. In a further attempt to eliminate the heat, slides were placed within dry Petri dishes seated on a flat ice surface. A film of water condensed even then. No sporidial tests were made by this method. Finally, one-half of a Petri dish was turned bottom side up on a flat cake of ice. Slides were then placed for exposure upon the glass surface, which was cooled by the imprisoned cool air held between the dish and the ice. No water film could be detected under these conditions,

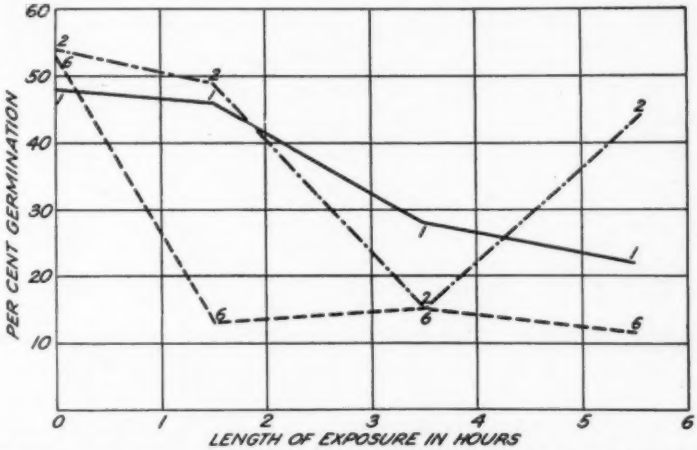

FIG. 14.—Comparison of the effect of exposure of sporidia of *Cronartium ribicola* for different periods at different saturation deficits. The figure at each point is the number of tests on which the point is based

Solid line: Saturation deficits between 2 and 2.9 mm.
Dot-dash line: Saturation deficits between 3 and 4.9 mm.
Dash line: Saturation deficits between 5 and 6.9 mm.

although the slides remained cool to the touch. Most of the heat was apparently eliminated without water condensing on the glass. In all of these tests, in order to eliminate the cooling from the ice as an additional factor influencing the germination of the exposed sporidia, some of those exposed without icing were, after exposure, iced for an equal length of time. Figure 15 shows the results for all tests, regardless of the length of exposure. All were low in germination, so the length of exposure within the periods in these experiments does not seem to be significant. The graph for the undried controls shows consistent higher germination at a fairly constant level than that which occurred in either the dried controls or the exposed slides. The dried controls show a rather high germination as compared with the exposed slides, but decidedly lower than the undried controls. The exposed sporidia gave less than 5 per cent germination, but continued to give this low percentage of germination at all saturation

deficits encountered in this series of experiments (5.0 to 22.5 mm.). The exposure to the sunlight was practically equally injurious through the entire series of experiments, regardless of the length of exposure or the saturation deficit at which they were exposed.

Figure 16 shows the results of certain experiments where exposure to sunlight was made for 30 minutes. The sunlight killed nearly all of the exposed sporidia, but not all, even at the large saturation deficit of 23 to 24 mm. Here again the preliminary drying as shown by the dried controls had a decidedly unfavorable effect on viability, as compared with the undried controls.

Figure 17 shows a comparison of the averages of the various kinds of sunshine exposures in three different experiments. In all three

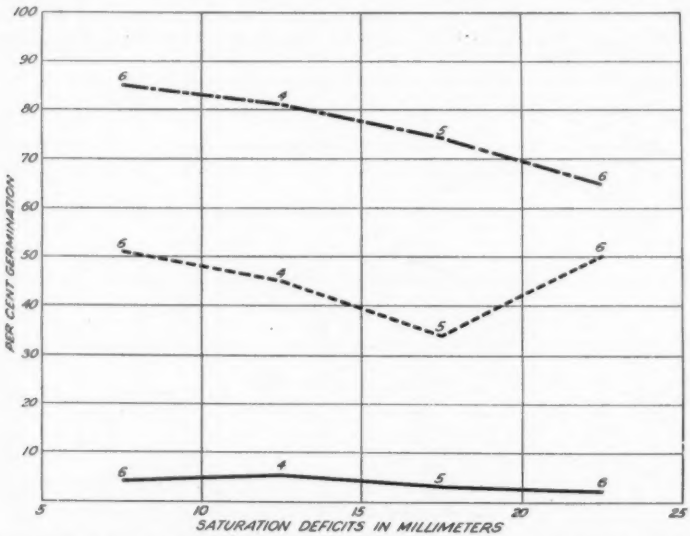

FIG. 15.—Effect of exposure of sporidia of *Cronartium ribicola* to direct sunlight at different saturation deficits. The figure above each point is the number of tests on which the point was based

Solid line: Germination on slides exposed to sunlight.
Dash line: Germination on comparable control slides which had received only the preliminary drying.
Dot-dash line: Germination on comparable control slides which had received neither the preliminary drying nor the subsequent exposure.

experiments the material was of approximately equal viability, as is clearly shown by the germination of the undried controls. These graphs show (1) that the preliminary drying decreased viability decidedly; (2) that exposure in the sunlight (on ice to eliminate heat) injured them still more; (3) that exposure in sunlight to full effect of light and heat caused still greater injury; and (4) that icing after exposure to sunlight, as in 3, had no perceptible stimulating effect; that is, the injury from the sunlight was so great that icing after exposure did not stimulate germination.

Finally, attention should be called to the remarkable persistence of viability in a few of the sporidia which were exposed to the rigorous conditions of complete and unaltered sunlight. That germination really occurred on these slides after they were exposed there can be

no doubt. For instance, at a saturation deficit of 9.0 mm. germ tubes of a maximum length of 50 μ were produced. Sporidia exposed at a saturation deficit of 15 mm. produced germ tubes with a maximum length of 30 μ, and also secondary sporidia. Those exposed at a saturation deficit of 19 mm. produced germ tubes up to 40 μ long. And after exposure at a saturation deficit of 24 mm. germ tubes 30 μ long were formed. The germ tubes of maximum length in the undried controls of the same series were of approximately the same length as these, or 20 μ shorter.

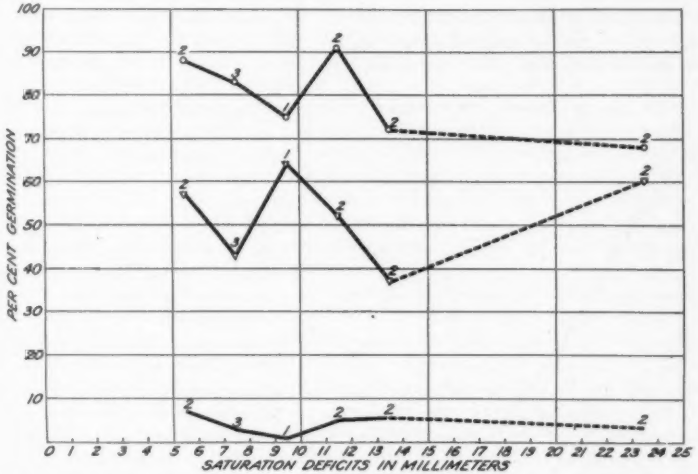

FIG. 16.—Effect of exposure of sporidia of *Cronartium ribicola* to sunlight for 30 minutes at various saturation deficits. The figure above each point is the number of tests on which the point was based. The broken sections of the lines indicate that the data were fragmentary

Solid line: Exposed to sunlight.
Circle solid line: Comparable undried controls.
Triangle solid line: Comparable dried controls.

EFFECT OF ICING ON VIABILITY OF SPORIDIA

During this study much difficulty was experienced in wetting dried and exposed slides with a film of water which was thin enough to give the best germination of the moistened sporidia. Spraying with an atomizer did not seem to give a continuous sheet of water which was thin enough for the best results under all conditions. Finally, upon the suggestion of L. H. Pennington, condensing moisture on the slides by putting them on ice was tried. The film of condensed moisture could be made heavier by prolonging the period of icing, but a perceptible continuous film could be obtained in one minute. Since a thin but continuous film of water was very desirable, this method of wetting the slides of dried sporidia was used in many of the experiments, in spite of the obvious undesirability of introducing an additional variable factor. The use of this method led to the incidental accumulation of data on the effect of icing upon the viability of the sporidia. In addition to this, some definite experiments were run under different conditions to determine whether icing really has an invigorating effect on the sporidia after they have

been weakened by adverse conditions. Ericksson (*25*, *26*) said that precooling seemed to stimulate and increase the germination of rust spores, but the present writers (*54*), in some preliminary tests of the effect of cooling upon the germination of the teliospores of *Cronartium ribicola*, found no evidence that precooling per se increased their germination.

For general comparison of the iced and uniced sporidia the results of eight groups of experiments were available. There were 54 pairs of slides in which the two were given identical treatments, except that one was iced just before germination, while the other was not. Twenty-six of the 54 iced slides produced secondary sporidia, whereas only 18 of the 54 uniced ones did so. The mean germination for the 54 iced slides was 27.2 per cent, and for the 54 uniced slides it was 17.6. This method of averaging gives excessive weight to the pairs of slides with better material.

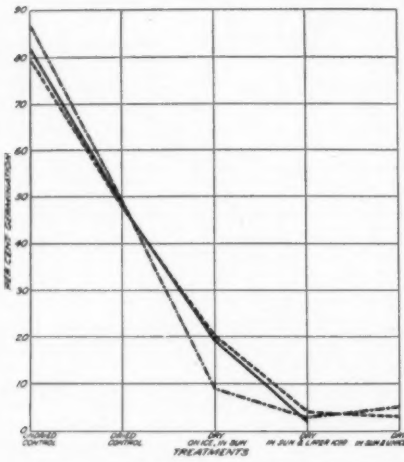

FIG. 17.—Comparison of the germination which followed different treatments of sporidia of *Cronartium ribicola* in three different experiments

Dot dash line: First experiment.
Solid line: Second experiment.
Dash line: Third experiment.

Another method of comparing the effect of cooling and noncooling which gives equal weight to all the individual comparisons is to consider the proportion of the comparisons in which the germination on the iced slides was better than that on the uniced ones. Of the 54 comparable pairs of slides the iced slide showed better germination in 35 pairs, and the uniced one in 17, while in 2 pairs none of the sporidia germinated on either slide. These last two pairs may be considered simply as tests in which a decision was prevented. In the 52 cases in which there was a difference between the iced and the uniced slides the most probable result, if the icing had had no effect, would have been superiority of the iced slides in half of the comparisons and of the uniced ones in the other half. In other words, the iced slides should have been expected to be superior in only 26 of the cases. The standard error of this expectation, proceeding on the assumption that p (the probability of the iced slide being best) equals q (the probability of the uniced one being best), and that each equals $\frac{1}{2}$, is found by the formula $(p \times q \times n)^{\frac{1}{2}}$ (*32*), which, in this case, is $(\frac{1}{2} \times \frac{1}{2} \times 52)^{\frac{1}{2}}$, or 3.6. The observed results that the iced slide was better in 35 of the cases differs from the expectation by 9, two and one-half times the standard error. If p and q are really equal as assumed, the odds would have been about 160 to 1 against the iced slide being superior to the uniced slide in as many as 35 cases. The odds, therefore, on the face of the results, are 160 to 1 that the assumption that icing is ineffective is an incorrect one. In other

words there is a rather high probability that icing did increase the amount of germination. The fact that the uniced slide was superior in so many cases would, on this basis, be taken as evidence that the effect of icing, while real, was less in magnitude than the variations due to fluctuations in sampling the sporidia or to some of the unknown and uncontrolled factors which made for variability.

Conclusions based on the above probability computations must be accepted with some reservations. Aside from the assumptions which must always be made in any application to biological data of methods based on the normal frequency curve, a further difficulty enters in, in that the population of comparisons was not a simple population. Some of the pairs had been exposed to drying or sunlight for a long time, some for a short time, and some not at all. Some were made at one time and some at another, some with material from one source and some material from another. It is thus entirely possible that some slides were affected differently from others; that in some tests the icing was beneficial, but that in slides which had received other types of preliminary treatment the icing was without effect or was even harmful. In any case, however, it is difficult to avoid the conclusion that the icing caused more germination on some slides.

To get information on whether icing affected both poor-germinating and well-germinating sporidia, the germination in each iced slide was averaged with its comparable uniced one. Then the population was divided into two groups, one group including the 27 pairs with the lowest average germination; and the other the 27 pairs with the highest average germination. The former group contained 17 pairs in which the iced slides germinated better than the uniced ones, 8 in which it germinated more poorly, and 2 in which there was no germination in either iced or uniced. In the latter group there were 18 pairs in which the iced slide germinated better, and 9 in which the uniced one germinated better. This indicates that despite the absence of true simple sampling, the population of the 52 pairs of slides was quite homogeneous, as far as reaction to icing is concerned.

Three groups of experiments were run to determine the effect of different periods of icing—ranging from 1 to 50 minutes in length—upon the germination of sporidia. These data were compared by these two methods: direct comparisons of the percentages of germination, and ranking by decrease in germination. The results in both methods are inconclusive. So far as these tests show, after 1 or 2 minutes there was no perceptible added effect from increasing the length of icing. In other words, a short period of icing (1 to 5 minutes) apparently has just as much stimulaive effect as a longer one.

If icing stimulates germination of sporidia, as it sometimes seems to do, then sporidia like those which Spaulding (52) found being produced on snow would be stimulated to increased germination and they probably could cause infections late in the season. There is a possibility that the apparent effect from icing was not a temperature effect but a moisture effect, since a thinner and more even film of water could be deposited by icing than by spraying.

In working up the above data no attempt was made to separate the effect of temperature from that of drying. There is a possibility that the temperature at which the sporidia were dried may have had an effect independent of that of drying. This temperature relation might account in part for the failure of most sporidia exposed by York at North Conway to germinate (*51*). This phase

of the problem should receive further investigation. Dole (*22*), in studying the effect of air temperature and relative humidity on the transpiration of *Pinus strobus*, found that "the products of actual losses and vapor pressure may be correlated with temperature, which means that temperature has an additional influence on the phenomenon other than its influence in determining the value of vapor pressure. This additional influence may be interpreted in terms of diffusion." If there were a separate temperature effect on the sporidia, it would probably be of the nature of a biochemical change in the protoplasm.

PRODUCTION OF SECONDARY SPORIDIA

Secondary sporidia are rather commonly produced by the various genera of the Uredinales (*2, 3, 4, 5, 6, 7, 8, 9, 16, 18, 28, 38, 40, 41, 43*). They were known by early workers (*43*) for some species of Cronartium, but Colley (*18*) seems to have been the first to report them for *Cronartium ribicola* in the literature.

Secondary sporidia were common in the experiments reported here (fig. 2), and the writers regarded them as an indication of vigorous sporidial germination. They were formed in small numbers on some slides, but usually they occurred in large numbers if at all. In a few cases tertiary sporidia were formed (fig. 2).

Fresh teliospores, as well as those which had been stored for some time, produced sporidia which in turn produced secondary sporidia when they germinated. Teliospores from *Ribes rotundifolium* stored out of doors in mosquito-netting bags for 21 days; from *R. americanum* stored for 38 days; from *R. triste* stored for 47 days; from *R. vulgare* stored for 49 days; from *R. nigrum* stored for 52 days and from *R. glandulosum* stored for 55 days, were all able to germinate and produce sporidia which were vigorous enough to in turn produce secondary sporidia.

Twenty-six of the iced slides in the 54 comparable pairs of iced and uniced slides produced secondary sporidia, whereas only 18 of the uniced ones did so. This suggests, but does not prove, that icing may stimulate the production of secondary sporidia.

Since Reed and Crabill (*40*) found that the sporidia of *Gymnosporangium juniperi-virginianae* produced secondary sporidia only when they were kept continuously wet from the time of their formation until the time of their germination, an attempt was made to ascertain the effect of drying upon the formation of secondary sporidia by *Cronartium ribicola*. All the strictly comparable pairs of undried control slides and slides subjected to preliminary drying were examined for the production of secondary sporidia. In 64 such pairs only one slide produced secondary sporidia. In 43 of the 64 pairs, the undried control produced the secondary sporidia, while the dried controls of but 21 pairs produced them. The significance of the apparent superiority of the undried controls in ability to produce secondary sporidia will be examined by the method employed on page 426. Assuming that the drying had no effect, the most likely result would be that in 32 of the pairs it would be the undried controls which produced secondary sporidia and that in 32 of the pairs it would be the dried controls. The standard deviation of the expected number is $\sqrt{\frac{1}{2}\times\frac{1}{2}\times 64}=4$. The difference between expected and observed results is 43−32, or 11, which is $2\frac{3}{4}$ times its standard

error. Then the odds are 330 to 1 that the preliminary drying really did decrease the ability of the sporidia to form secondary sporidia.

Besides these 64 pairs, there were 43 pairs in which neither slide produced secondary sporidia, and 75 pairs in which both produced them. These were cases in which the material was too poor or too good to give differential results by the rather crude criterion employed, so they probably may be disregarded. In other words, 65 per cent of the undried slides produced secondary sporidia, whereas only 53 per cent of the comparable slides which had received the preliminary drying did so.

Long exposure of sporidia in an air-dried condition did not prevent the formation of secondary sporidia; in fact, in 1923 the exposed sporidia produced secondary sporidia just as readily as those which received only the preliminary dryings. Some of the sporidia from *Ribes nigrum* which had been alternately wetted and dried 11 times produced secondary sporidia. For sporidia from each of the eight Ribes hosts the maximum exposure in an air-dried condition, after which secondary sporidia were produced, were as follows: *Ribes americanum* 5 hours; *R. cynosbati* 10 hours; *R. glandulosum* 10 hours; *R. nigrum* 26 hours; *R. odoratum* 1 hour; *R. rotundifolium* 3½ hours; *R. triste* none; and *R. vulgare* none. Few tests were made with the last two species, and it is probable that further tests would have shown that exposed sporidia from even these two could produce secondary sporidia. If, as Clinton and McCormick (*16*) suggests, "this formation of a secondary sporidium is probably to tide it over unfavorable conditions of infection," sporidia of *Cronartium ribicola*, which in turn produced secondary sporidia, are potentially dangerous to pines for much longer periods than the longevity of the primary sporidia would indicate.

The facts that teliospores which have been stored for a long period can produce sporidia which are vigorous enough to produce in turn secondary sporidia, and that sporidia exposed dry for some time can produce secondary sporidia, lengthen the time during which a given generation of sporidia is potentially capable of causing infection.

SUMMARY

The present investigation is the first attempt to make a detailed physiological study of the sporidia of any species of Cronartium. Such a study was undertaken with *C. ribicola*, because it is impossible to understand the nature and conditions of pine infection without a knowledge of the infecting sporidia.

The influence of physical factors, particularly drying, upon the sporidia from eight Ribes hosts, has been studied. Methods for making studies of this type have been developed.

The time necessary for the production of sporidia increased directly with the length of storage of the telial material. Temperature also influenced the time necessary for sporidial production. Sporidia appear to need water for germination, but the thinnest film is sufficient.

The viability of wet sporidia was decreased by merely bringing them to an air-dried condition and immediately rewetting them. The amount of injury increased directly with the increase in the saturation deficit of the air in which they were dried. This injury was shown in most cases by the reduction in the germination percentages rather than in vigor of growth.

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A few sporidia were able to survive as many as 11 alternate dryings and wettings, but their ability to germinate tended to decrease with each successive drying. When the atmospheric saturation deficits at which the successive dryings occurred were less than 4 mm. the injury to the viability of the sporidia was less than when they were more than 5 mm.

In desiccators where the relative humidity of the air was controlled by sulphuric acid of known specific gravities, the viability of the sporidia decreased directly with increase in the saturation deficits of the air. No injury from acid fumes was detected.

Sporidia from *Ribes americanum*, *R. cynosbati*, *R. glandulosum*, *R. nigrum*, *R. odoratum*, *R. rotundifolium*, *R. triste*, and *R. vulgare* survived exposures of nine or more hours in an air-dried condition. Some air-dried sporidia from *R. nigrum* survived exposure for 26 hours (the longest period tested) in air with saturation deficits between 2.2 and 3.4 mm. The viability of the sporidia tended to decrease with increases in the length of exposure and with increases in the saturation deficits of the air in which exposure occurred.

The thin continuous film of water obtained by icing the slides was most favorable for the germination of the sporidia. Precooling (icing) appeared to somewhat stimulate the germination of sporidia. Iced sporidia were less injured by successive dryings than were uniced ones. The length of the period of icing (if not less than one minute) appeared to be immaterial.

Small percentages of the sporidia survived short exposures in full sunlight with saturation deficits up to 23.5 mm.

Sporidia from all the tested species of Ribes produced secondary sporidia. Sporidia which were kept constantly wet appeared to produce secondary sporidia somewhat more readily than did the sporidia which had been dried.

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BIOLOGY OF THE SAW-TOOTHED GRAIN BEETLE, ORYZAEPHILUS SURINAMENSIS LINNÉ[1]

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INTRODUCTION

The saw-toothed grain beetle, *Oryzaephilus surinamensis* Linné, one of the best known of the insects that attack stored foods, is cosmopolitan in distribution and is likely to be found in almost any stored food of vegetable origin. Although it has been known to scientists for more than 150 years, the statement by Chittenden (*8, pp. 16–17*)[2] that "during the warmest summer months the life cycle requires but 24 days; in early spring, from 6 to 10 weeks" is practically the extent of the information that the present writers have been able to find in the literature on the biology of this important pest.

HISTORICAL

Naturalists probably were familiar with this beetle long before its description by Linné in 1767 (*25*). Redi (*29, Table XVII*), in 1671, mentioned and figured an insect which resembles *Oryzaephilus surinamensis*, and which Wheeler (*36*) considers to be this or a closely allied species. Linné received specimens of this insect from Surinam (Dutch Guiana) and for that reason gave it the specific name *surinamensis*. Comparatively little seems to have been written by the early scientists on the biology of this insect. Westwood (*35, p. 153*) reported in 1839 that he had discovered it in sugar, and stated in 1848 that he had observed both larvae and adults floating in tea or coffee sweetened with infested sugar.

In 1846 there appeared in the Cultivator (*32*) an account of the experiments of a Mr. Rich of Shoreham, Vt., against an insect that was infesting his mill. He found that fumigating with brimstone smoke and tobacco smoke had little effect, but that the liberal application of boiling water and whitewash destroyed the insects. According to Gavit (*17, pp. 661–662*) the insect was the saw-toothed grain beetle, and a short account of it by him, with illustrations of the different stages, was published in 1849.

The same year Blisson (*5*) gave a detailed description of the immature stages, with brief notes on the habits and transformations of the insect. In 1853 Perris gave a brief account of the habits of the larva (*28, pp. 628–631*). Washburn (*33*) conducted a large series of experiments with this insect, and in 1890 he reported his results, with recommendations for control. Chittenden, in 1896, gave brief notes on the habits of the beetle (*8, pp. 16–17; 9, pp. 121–122*). He found that the life cycle required from 6 to 10 weeks in early spring, and about 24 days in midsummer in the vicinity of Washington, D. C. Dean (*10, pp. 202–204*) obtained similar results in Kansas,

[1] Received for publication Feb. 2, 1926; issued September, 1926.
[2] Reference is made by number (italic) to "Literature cited," p. 451.

stating that the pupal stage required from 6 to 12 days and that in Kansas there were from four to six generations annually.

SYNONYMY

Linné, whose description of the insect in 1767 seems to be the earliest in the literature, named it *Dermestes surinamensis* (*25, p. 565*). Its cosmopolitan distribution and varied food habits attracted the attention of many scientists, and in the next few years after Linné's account it was redescribed under several different names. Fabricius (*14, p. 62*), in 1775, described it as *Anobium frumentarium*. De Geer (*18, p. 54*) the same year placed it in another genus, referring to it as *Tenebrio surinamensis*. Olivier (*26, no. 18, p. 10*), in 1790, referred to it as *Ips frumentaria;* and Fabricius (*15, pt. 2, p. 496*), in 1792, placed it in still another genus, referring to it as *Colydium frumentarium* and giving as synonyms all of the aforementioned names. In the same year (1792) Fabricius described the same species under the names *Dermestes sexdentatus* (*15, pt. 1, p. 232*) and *Scarites cursor* (*15, pt. 1, p. 96*). Kugelann in 1794 (*24, p. 566*) referred to it as *Lyctus sexdentatus*, Paykull in 1800 as *Colydium sexdentatum* (*27, p. 313*), and Gyllenhal in 1813 (*21, p. 406*) as *Silvanus sexdentatus*. In 1830 Stephens (*30, p. 104*) followed Gyllenhal in placing the species in the genus Silvanus but used the specific name *surinamensis*, and from that time until very recently it has been known to economic entomologists as *Silvanus surinamensis*. Ganglbauer in 1899 (*16, pp. 583–584*) revised the old genus Silvanus and placed the species *surinamensis* in the new subgenus Oryzaephilus. This revision has been accepted by most entomologists, and the saw-toothed grain beetle is now known as *Oryzaephilus surinamensis*.

Oryzaephilus surinamensis Linné:
Dermestes surinamensis Linné, 1767, Syst. Nat. ed. 12, 1 (2): 565.
Tenebrio surinamensis De Geer, 1775, Mém. Ins., 5 : 54, pl. 13, fig. 12.
Anobium frumentarium Fabricius, 1775, Syst. Ent. 1 : 62.
Ips frumentaria Olivier, 1790, Ins. 2 (18):10, pl. 2, fig. 13.
Colydium frumentarium Fabricius, 1792, Ent. Syst. 1 (2): 496.
Dermestes sexdentatus Fabricius, 1792, Ent. Syst. 1: 232.
Scarites cursor Fabricius, 1792, Ent. Syst. 1: 96.
Lyctus sexdentatus Kugelann, 1794, Schneid. Mag. 1: 566.
Colydium sexdentatum Paykull, 1800, Faun. Suec. 3: 313.
Silvanus sexdentatus Gyllenhal, 1813, Ins. Suec. 1 (3): 406.
Silvanus surinamensis Stephens, 1830, Ill. Brit. Ent. Mandibulata 3: 104.
Oryzaephilus surinamensis Ganglbauer, 1899, Käfer 3 (2): 584.

LOSSES

Losses caused by the saw-toothed grain beetle are often considerable. Dried fruit (fig. 1) stored for considerable periods before packing invariably becomes infested, and in addition to a direct loss in actual destruction of fruit an expense is incurred in eliminating the insect in the process of preparing the fruit for packing.

The damage done by the beetle to stored seeds is often considerable, although its attack usually follows that of other insects such as the rice weevil. Adults confined with only sound wheat invariably die from starvation. Yet the saw-toothed grain beetle, often called the "bran bug" by grain dealers, has sometimes proved to be the chief offender in grain shipments, particularly to grain held in and shipped from the Northwest.

The presence of the saw-toothed grain beetle in foodstuffs, particularly in cereal products such as flour, meal, and breakfast foods, causes loss by rendering the food unsalable or unpalatable. The beetle's flattish form allows it to penetrate apparently tightly wrapped

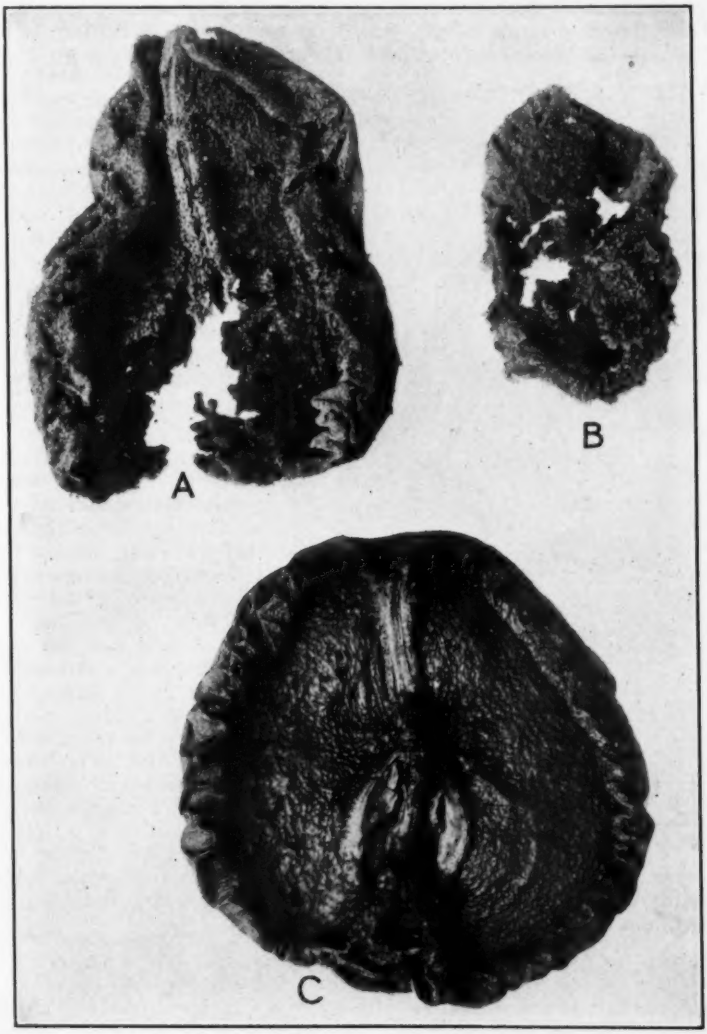

FIG. 1.—A and B, dried pears damaged by *Oryzaephilus surinamensis;* C, a normal dried pear

packages. The writers visited one grocery store that was overrun with this beetle and found that it had penetrated, with few exceptions, every box of crackers, breakfast cereals, and other packaged foods in the store. The presence of the beetles in so many com-

modities resulted in a rapid falling off of 50 per cent of the daily sales, and finally in a complete suspension of operations. The infestation in this case came from a barrel of scratch feed stored in a back room. The beetles multiplied so rapidly that they migrated to all parts of the store. This migratory habit was observed by Taschenberg (*31, pp. 19–20*) nearly 50 years ago. According to his account, the beetles became so numerous in a brewery that on warm days they invaded the neighboring houses, even crawling into the beds and nipping the occupants at night. A correspondent reported in 1922 from Seattle, Wash., that adults migrated in hordes from a feed store adjoining the residence and so overran the pantry and living rooms that nothing but the coming of cold weather ended the difficulty.

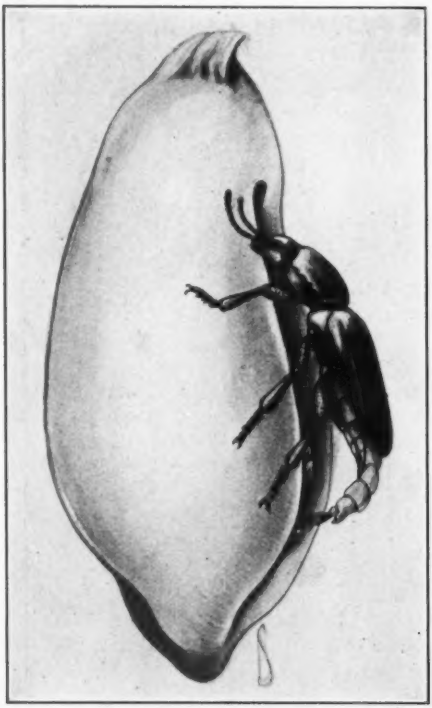

FIG. 2.—*Oryzaephilus surinamensis:* Female ovipositing on kernel of wheat. Note extended ovipositor and three eggs already deposited. All greatly enlarged

BIOLOGY

In the vicinity of Washington, D. C., the insect passes the winter in the adult stage, breeding normally ceasing late in the fall and commencing again in the spring. In heated buildings, if the relative humidity is not too low, breeding will continue at a slow rate throughout the winter. Most heated buildings, however, are so dry that little or no breeding takes place, although the adults remain active throughout the winter. Beetles kept during the winter in an incubator, in which the relative humidity was high, bred more or less freely. In tropical climates breeding undoubtedly continues throughout the year.

THE ADULT

The adult is a very active, slender, flattish, brown beetle, about one-tenth of an inch long (fig. 2). It has well developed wings, but rarely if ever flies. The posterior femora of the males, as Guillebeau (*20, p. 220*) has pointed out, are each armed with a tooth, which serves as an excellent character for distinguishing the males from the females, the femora of which are unarmed. The adult is nearly omnivorous, and many entomologists have published accounts of its

food habits. In this connection, Wheeler (*36*) very aptly states, "It is cosmopolitan and gregarious; living in nearly all stored human foods of vegetable origin; cereals (rice, wheat, maize, barley, etc.), ground or unground or in the form of paste (macaroni), bread etc.; dried fruits, nuts, copra; more rarely in sugar, starch, drugs, tobacco, snuff, or dried meats."

LONGEVITY

The beetle is rather long-lived. One specimen reared in the laboratory lived for 3 years and 3 months. This specimen was a male that transformed July 6, 1921. It was placed with a female for breeding records, but the female died shortly thereafter and the male was kept segregated for the rest of its life. Several other males reared in the laboratory lived for more than 2 years. Of the females reared in the laboratory, a majority of them lived for from 6 to 10 months, a few lived longer than 1 year, but only one lived for more than 2 years. This last-mentioned individual lived for 2 years and 8 months. All of these females were mated and they laid a considerable number of eggs. Data on the longevity of 10 females are given in Table 1.

TABLE 1.—*Data on the oviposition and longevity of 10 females of Oryzaephilus surinamensis, at Washington, D. C.*

No.	Date emerged	Date first egg was laid	Length of preoviposition period	Date last egg was laid	Length of oviposition period	Number of eggs laid	Date of death	Length of life
	1921	1922	*Days*	1922	*Days*			*Days*
1	Aug. 31	Mar. 26	207	June 26	92	226	July 1, 1922	304
2	Oct. 8	Apr. 2	176	Aug. 11	131	117	Aug. 14, 1922	310
3	Nov. 3	Apr. 1	149	June 17	77	167	Aug. 2, 1922	272
4	Nov. 7	do	145	Aug. 24	145	239	Jan. 17, 1923	436
5	do	Mar. 18	131	Aug. 7	142	103	Nov. 17, 1922	375
6	Dec. 21	Mar. 24	93	July 19	117	96	July 28, 1922	219
	1922							
7	Feb. 12	do	40	Aug. 14	143	285	Dec. 15, 1923	671
8	Feb. 15	Mar. 22	35	do	145	216	Oct. 8, 1924	966
9	Mar. 20	Apr. 2	13	July 18	107	45	Aug. 7, 1922	140
10	May 30	June 7	8	Aug. 28	82	235	Aug. 20, 1923	447

OVIPOSITION

The eggs are laid singly or in small clusters, and are usually tucked away out of sight in some crevice in the food supply selected or laid loosely in finely ground foods such as flour or meal. In obtaining egg-laying records in the laboratory, split kernels of corn were furnished as food and as repositories for eggs. When only one or two pieces of corn were supplied it was an easy matter to obtain the daily egg record.

Oviposition was repeatedly observed. In one typical instance the female maneuvered around a broken kernel of corn, feeling here and there with her ovipositor, apparently seeking a crevice in which to deposit her egg. After several minutes, during which she explored all of the kernel, she finally settled on a small crevice in the germ part, and into this crevice she thrust her ovipositor and remained thus for three minutes. Examination later showed the egg neatly tucked away in the crevice.

PREOVIPOSITION PERIOD

Individuals begin depositing eggs as soon as 5 to 7 days after emergence. Oviposition was repeatedly observed to have occurred this soon after emergence during hot midsummer weather, when large numbers of eggs were being obtained. The length of the preoviposition periods of 10 females are given in Table 1. The length varies greatly with the season of the year, and in these cases in Table 1 it ranged from 8 days as a minimum to 207 days as a maximum. Beetles emerging in the fall at Washington, D. C., rarely oviposit before the following April; females emerging in the spring at Washington normally have a preoviposition period of from 10 to 14 days, depending upon the prevailing temperature.

LENGTH OF OVIPOSITION PERIOD

As indicated by the data of Table 1, the oviposition period is quite long, ranging from about two to five months. The two longest periods were each of 145 days' duration—from March 22 to August 14 in one case, and from April 1 to August 24 in the other.

RATE OF OVIPOSITION AND TOTAL NUMBER OF EGGS DEPOSITED

The number of eggs normally produced on the days when oviposition took place was from 1 to 4, as indicated by the data of Table 2. Occasionally as many as 6 or 8 eggs were laid by a single female in 24 hours. The total number of eggs laid by individuals ranged from 45 to 285 (Tables 1 and 2).

THE EGG

The egg is white, shiny, and elongate-oval in form. In length it measures 0.83 mm. to 0.88 mm. and in width 0.25 mm. (fig. 3, A). The young embryo when fully developed nearly fills the eggshell, which becomes wrinkled and undulates with the movements of the embryo. When ready to break from the shell, the embryo sets in motion a series of undulations from caudad forward, the head and prothorax seeming to swell up at the end of each undulation, stretching the shell of the egg to the bursting point. Finally the shell breaks at the head and the undulating movements carry the young larva gradually clear of it.

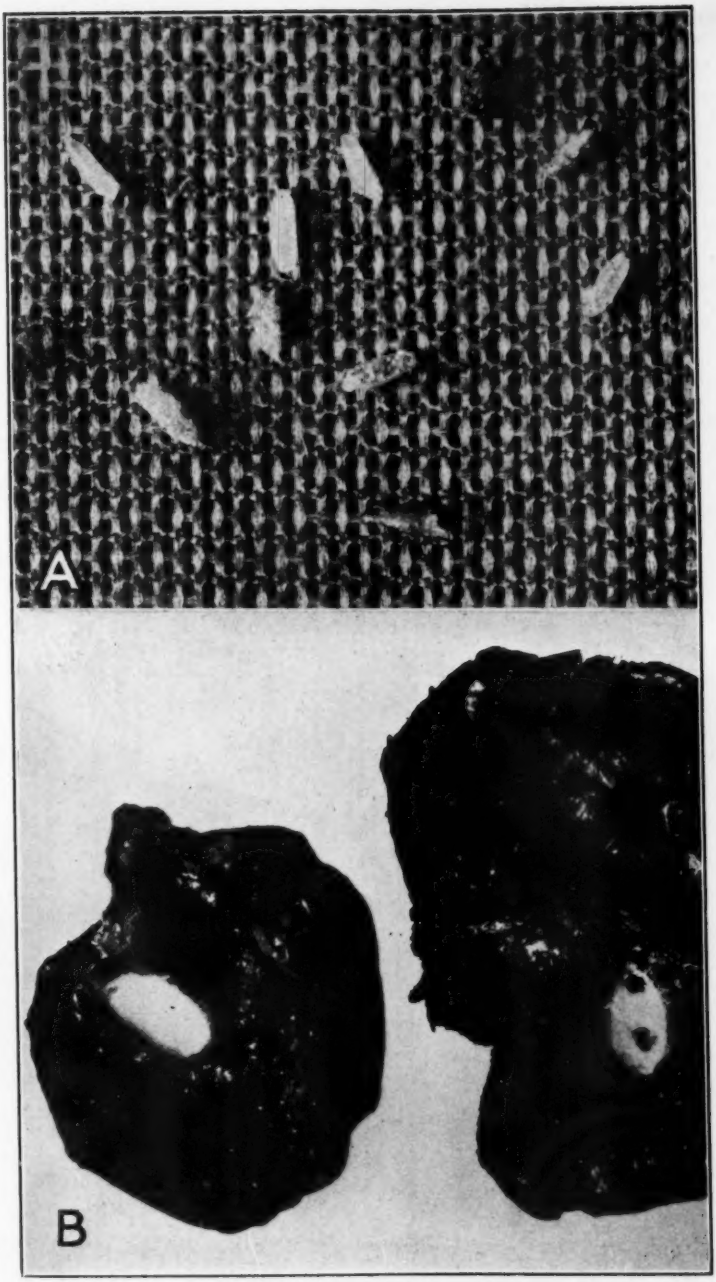

FIG. 3.—A, eggs of *Oryzaephilus surinamensis*, × 16 (photographed on No. 10 XX flour bolting cloth); B, white cocoons, greatly enlarged, formed by the larval parasite *Neoscleroderma tarsalis* (Ashm.)

TABLE 2.—*Daily oviposition records of 10 female of Oryzaephilus surinamensis*

Date	Number of eggs laid by individual No.—									
	1	2	3	4	5	6	7	8	9	10
1922										
Mar. 18	0	0	0	0	1	0	0	0	0	0
19	0	0	0	0	2	0	0	0	0	0
20	0	0	0	0	2	0	0	0	0	0
21	0	0	0	0	1	0	0	0	0	0
22	0	0	0	0	1	0	0	1	0	0
24	0	0	0	0	1	1	1	1	0	0
26	1	0	0	0	1	2	1	2	0	0
27	0	0	0	0	1	1	1	1	0	0
28	1	0	0	0	1	2	1	1	0	0
29	0	0	0	0	1	2	1	2	0	0
30	2	0	0	0	1	0	1	2	0	0
31	2	0	0	0	1	0	0	1	0	0
Apr. 1	1	0	1	1	2	0	2	2	0	0
2	1	1	1	0	2	1	1	2	1	0
3	2	0	2	2	1	0	1	0	0	0
4	2	1	0	2	2	2	2	2	1	0
5	2	2	0	2	1	0	2	0	0	0
6	2	2	2	2	2	2	1	1	0	0
7	2	2	2	2	2	1	2	1	1	0
8	3	1	1	1	1	0	1	0	0	0
9	2	0	2	1	2	0	2	0	0	0
10	3	1	3	2	3	2	2	2	1	0
11	2	2	2	1	2	1	2	0	0	0
12	2	2	1	2	2	0	2	0	1	0
13	3	2	2	2	2	1	1	0	1	0
14	4	2	2	1	2	1	3	1	0	0
15	3	0	2	2	0	0	0	0	0	0
16	1	0	2	2	0	1	1	0	1	0
17	1	0	2	2	0	1	1	2	1	0
18	0	1	3	2	0	2	2	1	1	0
19	0	1	3	1	0	1	1	0	0	
20	0	0	2	1	0	0	1	0	0	0
21	0	0	1	0	0	0	0	2	0	0
22	0	0	1	0	0	0	0	0	0	0
23	0	0	1	0	0	2	0	0	0	0
24	0	0	2	0	0	0	1	0	0	0
25	2	0	1	0	0	2	1	2	0	0
26	1	0	1	2	0	0	3	0	0	0
27	2	0	2	2	0	0	4	0	2	0
28	2	0	1	3	0	0	3	2	1	0
29	3	0	1	1	0	0	3	0	1	0
30	4	0	2	4	0	1	4	2	0	0
May 1	5	0	3	3	1	2	4	2	0	0
2	4	0	3	2	0	1	3	2	0	0
3	4	0	2	3	0	1	3	2	0	0
4	2	2	5	2	2	0	4	2	0	0
5	3	2	5	1	2	0	5	1	1	0
6	0	1	2	0	3	0	5	1	0	0
7	1	1	3	1	4	0	6	1	0	0
8	1	1	3	1	3	1	6	1	0	0
9	0	1	3	2	2	0	4	1	1	0
10	1	0	3	1	3	1	4	1	1	0
11	2	1	3	3	2	1	6	2	2	0
12	3	1	3	3	2	1	6	3	1	0
13	2	1	3	3	2	0	6	3	1	0
14	2	0	3	3	2	1	6	3	1	0
15	2	1	3	3	2	1	6	3	1	0
16	3	2	1	3	2	2	8	2	1	0
17	3	1	1	4	1	1	8	3	1	0
18	3	0	3	3	0	1	4	2	2	0
19	3	0	4	3	0	1	4	2	2	0
20	2	2	2	3	0	1	5	2	2	0
21	3	0	3	3	0	1	3	1	0	0
22	3	0	1	2	0	1	4	3	1	0
23	3	0	3	2	0	1	2	2	1	0

TABLE 2.—*Daily oviposition records of 10 females of Oryzaephilus surinamensis*—Continued

Date	Number of eggs laid by individual No.—									
	1	2	3	4	5	6	7	8	9	10
May 24	3	0	3	1	1	1	4	2	0	0
25	3	0	3	3	0	1	2	1	2	0
26	3	0	3	2	0	1	3	3	0	0
27	2	0	1	2	1	1	3	2	1	0
28	3	0	3	1	1	1	2	2	1	0
29	2	0	3	3	0	1	3	1	1	0
30	2	0	2	1	0	1	1	2	0	0
31	2	0	3	1	0	1	4	2	1	0
June 1	3	0	3	2	0	1	3	2	0	0
2	2	0	3	3	0	1	2	2	1	0
3	2	0	3	2	0	1	3	2	0	0
4	3	0	3	2	0	1	3	2	1	0
5	4	0	2	3	0	1	2	2	0	0
6	4	3	4	4	0	0	4	4	0	0
7	4	3	4	4	0	0	4	4	0	1
8	5	0	2	4	0	1	4	0	0	0
9	6	0	2	4	2	1	4	0	1	1
10	6	0	1	4	0	2	4	2	0	1
11	5	1	1	4	0	2	4	1	0	1
12	6	2	1	4	0	2	4	2	0	2
13	2	2	1	2	0	2	2	0	0	1
14	2	0	1	2	0	2	3	0	0	1
15	2	2	0	2	0	0	2	0	0	1
16	3	2	1	2	1	1	2	1	0	1
17	2	2	1	3	0	0	2	2	0	2
18	4	0	0	2	0	0	4	2	1	1
19	5	0	0	2	0	0	4	1	0	2
20	4	0	0	2	0	0	4	1	0	2
21	5	2	0	1	0	0	2	2	1	1
22	4	1	0	2	0	0	2	1	1	1
23	3	2	0	1	0	0	1	1	0	1
24	3	2	0	1	0	0	1	1	0	1
25	3	2	0	1	0	2	1	1	0	1
26	3	2	0	1	0	1	1	1	0	1
27	0	3	0	2	0	2	1	4	0	1
28	0	2	0	2	0	1	1	4	0	1
29	0	0	0	3	0	2	0	1	0	1
30	0	0	0	4	0	2	1	2	0	1
July 1	0	0	0	5	0	2	0	1	0	1
2	0	0	0	4	0	2	1	5	0	6
3	0	0	0	4	0	2	1	6	0	6
4	0	0	0	1	0	0	0	4	0	2
5	0	0	0	1	0	0	1	3	0	3
6	0	0	0	1	0	2	0	1	0	3
7	0	0	0	1	0	0	0	1	0	3
8	0	0	0	1	0	0	0	2	0	3
9	0	3	0	1	2	0	0	1	0	3
10	0	2	0	2	2	0	0	2	0	3
11	0	4	0	3	2	0	0	1	0	4
12	0	3	0	3	1	0	1	3	0	4
13	0	2	0	2	0	0	0	2	0	5
14	0	1	0	2	0	2	0	2	0	5
15	0	2	0	1	0	0	0	1	0	2
16	0	2	0	1	1	0	0	1	0	2
17	0	2	0	1	0	1	0	1	0	2
18	0	2	0	3	0	0	0	3	1	4
19	0	2	0	3	0	1	1	1	0	3
20	0	0	0	1	0	0	3	4	0	4
21	0	0	0	1	0	0	3	2	0	4
22	0	1	0	1	2	0	0	1	0	6
23	0	1	0	1	2	0	0	1	0	7
24	0	1	0	1	2	0	0	2	0	7
25	0	0	0	2	0	0	0	1	0	5
26	0	0	0	2	0	0	0	1	0	5
27	0	0	0	1	1	0	1	1	0	3

TABLE 2.—*Daily oviposition records of 10 females of Oryzaephilus surinamensis*—Continued

Date	Number of eggs laid by individual No.—									
	1	2	3	4	5	6	7	8	9	10
July 28	0	0	0	1	1	0	0	2	0	3
29	0	0	0	0	0	0	0	0	0	5
30	0	0	0	0	1	0	0	1	0	5
31	0	0	0	1	0	0	0	0	0	5
Aug. 1	0	0	0	0	1	0	1	2	0	4
2	0	0	0	0	1	0	0	0	0	4
3	0	3	0	0	1	0	0	2	0	4
4	0	2	0	0	1	0	0	1	0	5
5	0	2	0	0	1	0	0	0	0	3
6	0	2	0	0	1	0	1	3	0	4
7	0	2	0	0	1	0	1	2	0	3
8	0	3	0	0	0	0	0	1	0	4
9	0	3	0	0	0	0	1	0	0	4
10	0	1	0	0	0	0	2	0	0	2
11	0	1	0	0	0	0	1	1	0	3
12	0	0	0	0	0	0	0	4	0	1
13	0	0	0	0	0	0	1	0	0	1
14	0	0	0	0	0	0	1	1	0	1
15	0	0	0	0	0	0	0	0	0	3
16	0	0	0	0	0	0	0	1	0	2
17	0	0	0	0	0	0	0	0	0	3
18	0	0	0	0	0	0	0	0	0	4
19	0	0	0	0	0	0	0	0	0	3
20	0	0	0	0	0	0	0	0	0	3
21	0	0	0	0	0	0	0	0	0	2
22	0	0	0	0	0	0	0	0	0	3
23	0	0	0	0	0	0	0	0	0	3
24	0	0	0	1	0	0	0	0	0	3
25	0	0	0	0	0	0	0	0	0	3
26	0	0	0	0	0	0	0	0	0	3
27	0	0	0	0	0	0	0	0	0	3
28	0	0	0	0	0	0	0	0	0	4
Total	226	117	167	239	103	96	285	216	45	235

LENGTH OF EGG STAGE

The length of the egg stage varies with the temperature, as is indicated by the data of Table 3. In midsummer, with the mean average temperatures ranging from about 80° to 85° F., the eggs hatch in from 3 to 5 days after deposition. In cool spring and fall weather, with the mean average temperatures ranging from 73° to 68°, the eggs hatch in from 8 to 17 days.

TABLE 3.—*Incubation period of egg of Oryzaephilus surinamensis*

No.	Date egg was laid	Date egg was hatched	Incubation period	Mean average temperature for period	No.	Date egg was laid	Date egg was hatched	Incubation period	Mean average temperature for period
	1921	1921	*Days*	°*F.*		1921	1921	*Days*	°*F.*
1	July 20	July 24	4	82	16	Oct. 20	Nov. 3	13	70
2	July 21	July 25	4	82	17	Oct. 24	Nov. 4	11	70
3	July 22	...do...	3	82	18	Nov. 10	Nov. 22	12	68
4	July 23	July 26	3	83	19	Nov. 13	Nov. 26	13	68
5	July 24	July 27	3	85	20	Nov. 18	Nov. 30	12	69
6	Aug. 1	Aug. 5	4	80	21	Nov. 23	Dec. 6	13	68
7	Aug. 2	Aug. 7	5	80		1923	1923		
8	Aug. 3	Aug. 8	5	80					
9	Sept. 12	Sept. 17	5	79	22	Mar. 1	Mar. 14	13	71
10	Sept. 25	Oct. 3	8	73	23	...do...	Mar. 16	15	71
					24	Mar. 5	Mar. 22	17	70
11	...do...	Oct. 5	10	72	25	...do...	Mar. 21	16	70
12	Sept. 29	Oct. 9	10	70	26	Mar. 7	Mar. 23	16	70
13	...do...	Oct. 11	12	70					
14	Oct. 2	Oct. 14	12	69					
15	Oct. 15	Oct. 27	12	70					

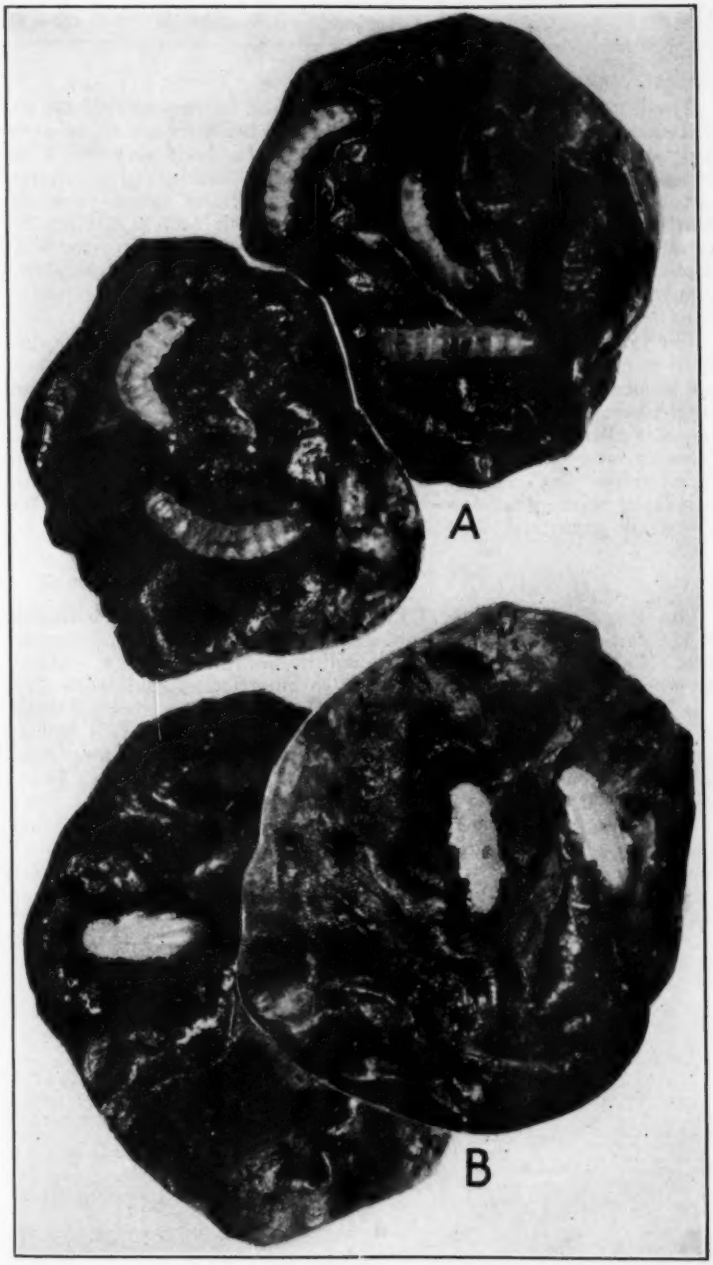

FIG. 4.—*Oryzaephilus surinamensis:* A, Five well-grown larvae on small seedless raisins; B, three pupae on seedless raisins. All greatly enlarged

THE LARVA

The larva is white when first hatched, and its movements are slow and uncertain. It soon gains strength and becomes active, however, and begins to attack the available food. Its body acquires a pale yellowish color, with darker bands on the dorsal surface of the thoracic and abdominal segments. Numerous long hairs adorn these segments. The head capsule is a pale yellowish brown. When first hatched the larva is about 0.80 to 0.90 mm. in length, and the width of the head capsule is 0.24 mm. When fully grown the larva attains a length of from 2.5 to 2.8 mm., and the width of the head capsule is from 0.46 mm. to 0.54 mm.[3] (Fig. 4.)

The larva thrives on practically all foodstuffs of vegetable origin, such as corn, wheat, barley, and rice, both ground and unground, and in all their varied forms, such as flour, meal, and breakfast foods; dried foods, nut meats, copra, etc. It is free-living and active, and does not always confine its feeding to one spot, but nibbles here and there as fancy dictates. It is apparently unable to feed on whole grain unless the grain is quite soft. It is therefore frequently associated with other insects, such as the rice weevil, which bore into whole grain and thus afford entry to the weaker species.

LENGTH OF LARVAL STAGE

The length of the larval stage as determined at Washington, D. C., varied considerably, and was affected chiefly by the temperature. Data bearing on larval life will be found in Table 4. During the spring the period from hatching to pupation ranged from about 4 to 7 weeks, whereas in midsummer this period was about 2 weeks, 12 days being the shortest larval period recorded. With the approach of cooler weather in the fall, the period again lengthened until during the winter months, when the laboratory was heated only during the day, the period was about 8 to 10 weeks.

The number of molts varied (Table 4.) A majority of the larvae observed molted three times, a few molted four times, and quite a number molted but twice. Those individuals that molted four times were all reared during the fall and winter, when development was slow and the larval period quite long.

[3] A technical description of the typical silvanid larva has been given by Böving (*6, pp. 201–203*).

TABLE 4.—*Life-history data on 30 individuals of Oryzaephilus surinamensis*

No.	Date egg was laid	Date hatched	Length of egg period	Date of first molt	Length of first larval stage	Date of second molt	Length of second larval stage	Date of third molt	Length of third larval stage	Date of fourth molt	Length of fourth larval stage	Date of beginning of prepupal stage	Length of last larval stage	Date of pupation	Length of prepupal stage	Date adult emerged	Length of pupal period	Length of larval period	Length of period, egg to adult	Mean average temperature for period
	1921	1921	*Days*	1921	*Days*	1921	*Days*	1921	*Days*	1921	*Days*	1921	*Days*	1921	*Days*	1921	*Days*	*Days*	*Days*	° *F.*
1	July 19	July 23	4	July 30	7	Aug. 3	4	Aug. 7	4			Aug. 9	2	Aug. 10	1	Aug. 20	10	18	32	80
2	July 20	July 24	4	...do	6	Aug. 1	2					Aug. 6	5	Aug. 8	2	Aug. 16	8	15	27	81
3	July 22	July 25	3	July 29	4	...do	3					Aug. 5	4	Aug. 7	2	Aug. 13	6	13	22	81
4	July 23	July 26	3	July 30	4	...do	2	Aug. 5	4			Aug. 8	3	Aug. 9	1	Aug. 17	8	14	25	80
5	...do	July 27	4	...do	3	Aug. 3	4	Aug. 7	4			Aug. 9	2	Aug. 10	1	Aug. 16	6	14	24	81
6	July 25	July 28	3	July 31	3	Aug. 4	4	Aug. 8	4			Aug. 10	2	Aug. 11	1	Aug. 19	8	14	25	80
7	July 26	July 30	4	Aug. 3	4	Aug. 7	4					...do	3	Aug. 12	2	Aug. 20	8	13	25	80
8	July 27	...do	3	...do	4	...do	4					Aug. 9	2	Aug. 11	2	Aug. 19	8	12	23	80
9	July 29	Aug. 1	3	Aug. 5	4	Aug. 10	5					Aug. 12	2	Aug. 16	4	Aug. 22	6	15	24	79
10	July 31	Aug. 3	3	Aug. 7	4	Aug. 12	5					Aug. 17	5	Aug. 19	2	Aug. 29	10	16	29	78
11	Aug. 1	Aug. 5	4	Aug. 9	4	Aug. 13	4	Aug. 17	4			Aug. 19	2	Aug. 20	1	Aug. 31	11	15	30	78
12	Sept. 12	Sept. 17	5	Sept. 25	8	Sept. 30	5					Oct. 9	9	Oct. 12	3	Oct. 25	13	25	43	73
13	Sept. 27	Oct. 6	9	Oct. 12	6	Oct. 28	16	Nov. 9	12	Nov. 25	16	Dec. 9	14	Dec. 14	5	1922 Jan. 2	19	69	97	69
14	Sept. 28	Oct. 9	11	Oct. 19	10	Oct. 29	10	Nov. 5	7	Nov. 20	15	Nov. 28	8	Dec. 1	3	1921 Dec. 20	19	53	83	69
15	Sept. 29	Oct. 11	12	Oct. 22	11	Oct. 30	8	Nov. 10	11			Nov. 29	19	Dec. 3	4	Dec. 21	18	53	83	69
16	Sept. 30	Oct. 12	12	...do	10	Nov. 2	11	Nov. 14	12	Dec. 1	17	Dec. 22	21	Dec. 26	4	1922 Jan. 16	21	75	108	69
17	Oct. 15	Oct. 27	12	Nov. 4	8	Nov. 18	14	Nov. 28	10	Dec. 8	10	...do	14	Dec. 29	7	Jan. 17	19	63	94	69
18	1923 Mar. 1	1923 Mar. 16	15	1923 Mar. 29	13	1923 Apr. 8	10	1923 Apr. 17	9			1923 Apr. 23	6	1923 Apr. 25	2	1923 May 9	14	40	69	70
19	Mar. 3	Mar. 19	16	Apr. 2	14	Apr. 10	8	Apr. 19	9			Apr. 26	7	May 1	5	May 16	15	43	74	70
20	Mar. 5	Mar. 21	16	Apr. 5	15	Apr. 12	7	Apr. 22	10			May 4	12	May 8	4	May 20	12	48	76	70
21	...do	...do	16	Apr. 3	13	Apr. 10	7	...do	12			May 1	9	May 5	4	May 17	12	45	73	70
22	Mar. 7	Mar. 23	16	Apr. 5	13	Apr. 14	9					Apr. 24	10	Apr. 28	4	May 14	16	36	68	70
23	Apr. 3	Apr. 16	13	Apr. 25	9	May 3	8	May 11	8			May 20	9	May 22	2	June 2	11	36	60	73
24	...do	Apr. 17	14	Apr. 24	7	May 1	7	May 9	8			May 17	8	May 20	3	May 30	10	33	57	73
25	Apr. 6	Apr. 19	13	Apr. 27	8	May 5	8	May 13	8			May 20	7	May 23	3	June 2	10	34	57	73
26	Apr. 5	Apr. 18	13	Apr. 26	8	May 8	12					May 19	11	May 21	2	May 31	10	33	56	73
27	Apr. 6	Apr. 19	13	Apr. 27	8	May 5	8	May 16	11			May 24	8	May 26	2	June 3	8	37	58	73
28	Apr. 7	Apr. 20	13	May 3	13	May 9	6	...do	7			May 22	6	...do	4	...do	8	36	57	73
29	...do	...do	13	Apr. 28	8	May 6	8	May 12	6			May 19	7	May 21	2	May 31	10	31	54	73
30	...do	...do	13	...do	8	May 5	7					May 15	10	May 18	3	May 28	10	28	51	73

THE PUPA

When the larva completes its growth it usually constructs a rude pupal cell or cocoon with particles of seeds or other foodstuffs, fastening them together with an oral secretion. At other times no pupal cell is made. In both cases the larva attaches itself by the anal end to some solid object. After a short prepupal period, during which time it is more or less quiescent, it transforms to the pupal form. (Fig. 3, B.) The pupa remains attached by its caudal end to the cast larval skin. Blisson (*5*) described this transformation in 1849.

For the individuals in Table 4 the prepupal period ranged from 1 to 7 days, and the pupal period from 6 to 21 days. (Table 5.)

TABLE 5.—*Effect of temperature on the length of the pupal stage of Oryzaephilus surinamensis, as indicated by 30 individuals*

No.	Date pupated	Date adult emerged	Length of pupal stage	Mean average temperature for period	No.	Date pupated	Date adult emerged	Length of pupal stage	Mean average temperature for period
	1921	1921	*Days*	°*F.*		1921	1922	*Days*	°*F.*
1	July 31	Aug. 7	7	81	16	Dec. 26	Jan. 16	21	68
2	Aug. 7	Aug. 13	6	77	17	Dec. 29	Jan. 17	19	68
3	Aug. 8	Aug. 16	8	76		1923	1923		
4	Aug. 10	Aug. 19	9	76.5	18	Apr. 23	May 8	15	72
5	Aug. 12	...do...	7	77	19	Apr. 25	May 9	14	72
					20	Apr. 27	May 13	16	72
6	Aug. 13	Aug. 20	7	78					
7	...do...	Aug. 19	6	78	21	Apr. 28	May 14	16	72
8	Aug. 16	Aug. 22	6	78	22	May 1	May 16	15	73
9	Aug. 19	Aug. 27	8	76	23	May 5	May 17	12	73
10	Oct. 12	Oct. 25	13	71	24	May 8	May 20	12	73
					25	May 18	May 28	10	75
11	Oct. 20	Nov. 2	13	70					
12	Nov. 30	Dec. 17	17	66	26	May 22	May 31	9	77
13	Dec. 1	Dec. 20	19	69	27	...do...	June 2	11	77
14	Dec. 3	Dec. 21	18	69	28	May 23	...do...	10	77
		1922			29	May 26	June 3	8	80
15	Dec. 14	Jan. 2	19	69	30	...do...	...do...	8	80

LENGTH OF LIFE CYCLE

The effect of temperature on the length of time required for the individual to pass through the egg, larval, and pupal stages is indicated by the following data derived from Table 4. In warm summer weather, when the mean average temperature for the period of development was 81° F., three individuals required 22, 24, and 27 days to pass through the egg, larval, and pupal stages. When the mean average temperature was 80° five other individuals required 23, 25, 25, 25, and 32 days. In the spring, when the mean average temperature was 70°, five other individuals required 68, 73, 76, 74, and 69 days. The longest period noted was 108 days (No. 16, Table 4), the mean average temperature for the period (September 30 to January 16) being 69°. Four other specimens required 83, 83, 94, and 97 days, at the same temperature. With the life of the adult ranging from a few days to 3 years and 3 months, the length of one generation varies greatly. Inasmuch as the preoviposition period has been found to range from 5 to 207 days and the period from egg to adult from 22 to 108 days, it seems safe to state that the cycle from egg to egg may range from 27 to 315 days. In the vicinity of Washington, D. C.,

there are normally from four to five generations a year, when one considers a generation as covering the period from the time of hatching to the laying of the first eggs by the emerging adult. In subtropical and tropical climates the breeding is undoubtedly continuous.

PARASITES

As far as the writers are aware, the only parasite of the saw-toothed grain beetle is a hymenopteran described by Ashmead (*1, p. 45*) as *Ateleopterus tarsalis* from specimens reared from larvae of *Oryzaephilus surinamensis* infesting raisins. He stated that adults had been reared in Indiana from *O. surinamensis* infesting grain. Washburn (*34, p. 205*) and Brues (*7, p. 610*) refer to this parasite as *Neoscleroderma tarsalis* (Ashm.) in their accounts of the Hymenoptera of Minnesota and Connecticut, respectively, but neither gives further information. The writers have reared this parasite from larvae of *O. surinamensis* infesting raisins at Washington, D. C., and in 1922 they received specimens reared in Texas by T. Remy. The cocoons spun by the larvae of the parasite are illustrated in Figure 2, B. It is doubtful whether this parasite is of practical value, except, perhaps, in the case of natural control, when its host is infesting grain and stock feeds held in storage for unusually long periods.

CONTROL MEASURES

A study with the object of devising control measures for the saw-toothed grain beetle is in progress, and it may be well to report some of the findings here.

STARVATION.—The beetles are not very resistant to starvation; adults kept without food in midsummer were all dead within five days.

HIGH AND LOW TEMPERATURES.—The insect is fairly resistant to low temperatures in all stages except the egg stage. De Ong (*12, p. 446; 13, p. 74*) stated that a three months' exposure at 10° to 36° F. would kill the larvae, pupae, and adults. The writers found that a small percentage of adults and larvae survived an exposure for a period of three weeks within a temperature range of 30° to 35° F. Exposure within a temperature range of 20° to 25° killed all stages in one week, and exposure to 0° to 5° killed all stages in one day.

All stages succumb readily to heat. Goodwin (*19*) found that a temperature of 111° to 113° F. was fatal to larvae, pupae, and adults, although he did not state what length of exposure was necessary. Dean (*10, p. 163*) has reported that no grain pest (including the saw-toothed grain beetle) can withstand a temperature of from 118° to 122° for any considerable length of time. The writers found that exposure for one hour to 125° was fatal to all stages.

FUMIGANTS.—Hinds (*23, pp. 20–21*) found that the saw-toothed grain beetle lived for about two hours in an atmosphere saturated with carbon disulphide at a temperature of 90° F. The writers (*2*) have indicated that the ethyl acetate-carbon tetrachloride mixture can be used successfully in killing this pest. As a rule, a heavier-than-air gas is more satisfactory in fumigating infested grain, grain products, and nut meats. Hydrocyanic-acid gas is used frequently and with success under certain conditions calling for no great penetration of infested bulks. Hamlin and Benton (*22*) have recently established the

practicability of fumigating stemmer trash at raisin-packing plants in California with hydrocyanic-acid gas generated from calcium cyanide flakes.

Bioletti (*4, p. 189*) recommends the following method for the disposal of this infested trash:

> By modifying the shape of the receptacle which receives the screenings, including insects, all the beetles could be caught and destroyed. The modification consists of an over-lapping removable border furnished with a pad saturated with coal-oil or phenol to prevent the escape of the beetles. At intervals this receptacle is emptied and the insects destroyed.

HERMETICALLY SEALING AND VACUUM.—Dendy (*11, p. 11*) has found that hermetically sealing 100 adult beetles in a 15 c. c. capacity test tube three-fourths filled with crushed wheat for two days at 31° C. (87.8° F.) resulted in their death. The writers (*3*) found that adults kept in a vacuum of 29 inches for seven hours were killed.

SUMMARY

The saw-toothed grain beetle, *Oryzaephilus surinamensis* Linné, is one of the best known of the cosmopolitan grain pests. It attacks in both its larval and adult stages all food of vegetable origin, especially grain and such grain products as flours, meals, breakfast foods, stock and poultry feeds, and copra, nut meats, candies, and dried fruits.

No detailed study of this pest seems to have previously been made. Adult beetles have been found to live for more than 3 years. The majority of ovipositing females live from 6 to 10 months. The length of the preoviposition period ranges from 5 to 8 days during the season most favorable for breeding, to 207 days when climatic conditions are less favorable. The total number of eggs laid by an individual has been found to range from 45 to 285.

Eggs hatched in from 3 to 5 days in midsummer. In cooler weather hatching may not have taken place until 8 to 17 days after deposition. Larvae required about 12 days for development, under favorable midsummer conditions. In the spring from about 4 to 7 weeks were required to pass the period from hatching to pupation. Under less favorable conditions larvae have required 10 weeks for development. Larvae molt from two to four times, the majority molting three times. Data presented indicate a prepupal period of 1 to 7 days, and a pupal period of 6 to 21 days.

Under the most favorable weather conditions, the egg, larval, and pupal stages may be passed in as few as 22 days. The longest period required for the same development was 108 days, when the mean average temperature for the period was 69° F. The life cycle from egg to egg may range from 27 to 315 days. At Washington, D. C., there are four or five generations annually; in subtropical and tropical climates breeding is undoubtedly continuous.

As far as the writer is aware, the only known parasite of the saw-toothed grain beetle is a small cocoon-making hymenopteron, *Neoscleroderma tarsalis* (Ashm.).

In the adult stage the saw-toothed grain beetle is quite resistant to fumigants, but it can be killed by fumigation with hydrocyanic-acid gas, carbon disulphide, ethyl acetate-carbon tetrachloride mixture, or any other effective fumigant. Although an exposure of from 0° to 5°

F. for one day will kill all stages, adults and 'arvae withstood for three weeks a temperature ranging from 30° to 35°. An exposure for one hour at 125° has killed all stages. A vacuum of 29 inches continued for seven hours has killed adults.

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CORN ROOT ROT—A SOIL-BORNE DISEASE[1]

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INTRODUCTION

The present situation in regard to the corn root-rot problem is well summarized by Holbert and others in the following statements by them in a recent publication (*4, p. 243*):[2] "The corn root, stalk, and ear rot diseases are a group of diseases seriously affecting the corn crop. For brevity, these diseases are sometimes called 'corn-rot diseases,' and frequently simply 'corn root rot.' However, at the outset it must be realized that 'corn root rot' is not one disease, but several diseases, some of which do not result in any rotting of either roots or stalks." Although it is realized that the problem is complicated by the fact that there are several diseases, sufficient attention has not been given by plant pathologists to an attempt to separate the various diseases concerned and to properly evaluate them.

Confusion has arisen mainly from the fact that in the earlier studies of the problem the relation between poor seed and seedling blights was emphasized. These seedling blights, if they did not kill the plants, stunted them so seriously that they were at a disadvantage throughout life, although they may have largely recovered from the initial injury. These earlier studies centered attention on seed-borne pathogens, nearly to the exclusion of a study of the organisms actually associated with the diseased corn roots. In much of the literature on corn root rot it appears that the delayed development following seedling injury has been considered as typical corn root rot. If these effects of seedling blight are to be considered corn root rot, the fact should be recognized. If, however, there is a true corn root rot other than seedling blight, the two diseases should be recognized clearly and a distinction should be made between them in future literature.

That there is a disease (or group of diseases) which should be known as corn seedling blight is evident from the abundant literature on seed selection and the corn root rot problem. The work of Hoffer, Holbert, and others shows clearly that seed known to be infected with *Gibberella saubinetii*, *Diplodia zeae*, or possibly others of the common corn ear pathogens, gives a crop inferior to one produced from apparently healthy seed. It has been shown that the diseased seeds often produce weak plants which may die prematurely or may be weak throughout their lives. This fact has been recognized for a long time by many careful farmers who have made it a practice to discard all ears which are likely to be affected with any of the true ear-rot organisms (*Diplodia zeae, Gibberella saubinetii*). By ear selection at harvest time and by curing it is possible to obtain ears for seed in which the factor of seedling blight is almost negligible.

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[2] Reference is made by number (italic) to "Literature cited," p. 475.

This does not mean that ears free from seed-borne organisms may be obtained by such means, but that seedlings are produced sufficiently vigorous to develop in spite of the presence of such common organisms as *Fusarium moniliforme* and *Rhizopus* sp., which in weak corn appear to be able to cause seedling blight by attacking the surface of the scutellum and thus cutting off the food supply of the young plant.

The elimination of the factor of seedling blight does not, however, in the writers' observation, in any way control root rot which appears later in the season. About the time of ear formation in Kentucky it is difficult, on corn grown in some long-cultivated fields, to find more than a few live roots, the plants appearing to subsist on moisture and plant food drawn from the soil largely through dead roots and new roots thrown out after rainy periods. On continuous-corn plots on the Kentucky Agricultural Experiment Station farm, on land kept in a high state of fertility, this condition is much more prominent than in plots in the longer rotations; the plants may make a vigorous early growth, but when nearing maximum growth they may show marked symptoms of root injury, as evidenced by wilting, curling, and premature death of the leaves, while the corn in near-by rotation plots with actually lower soil moisture may not be wilting. This condition appears to the writers to be that which should be recognized as corn root rot in contrast to seedling blights and poor development due to adverse soil conditions.

With regard to the identity of the supposed causes of corn root rot as it is generally understood, there seems to be some unanimity of opinion in the recent literature. Manns and Phillips (*6*), following a review of recent literature, state:

> The literature as here reviewed shows that investigators have determined upon four different organisms as the principal ones associated with corn-rot diseases. These organisms are *Fusarium moniliforme* Sheldon; *Gibberella saubinetii* (Mont.) Sacc.; *Diplodia zeae* (Schw.) Lév., and *Cephalosporium sacchari* Butler. It is known that several of these organisms inhibit germination and appear to be active factors in the production of stalk and ear rots. Their importance as factors in causing root rot, however, is still in dispute. It is even questioned whether soil reaction is not the real factor predisposing corn to attacks of these so-called seedling and root-rot diseases.

Tehon (*8*) states that "Root and stalk rots of corn are chiefly caused by the fungi *Fusarium moniliforme*, *Diplodia zeae*, and *Gibberella saubinetii*. The degree of injury done by these organisms is dependent upon the relative susceptibility of the corn plant in the various stages of its development, and the effects of the attack appear as rotted roots, barren stalks, or poorly filled ears."

Holbert (*4*) and his coworkers apparently are in accord with these statements in regard to the causes of corn root rot, as their principal line of attack has been a study of the seed-borne organisms and their effect on the seedling plant and its subsequent development. Their work deals primarily with *Gibberella saubinetii* and *Diplodia zeae* and the effect of the various environmental factors on development of plants from seed infected and not infected by these organisms. They recognize the importance of soil infection in the problem, for they say: "Of the environing factors affecting the development of the different corn-rot diseases under discussion in this bulletin, the problem of *crop rotations* and *crop sequence* takes a place second to no other under conditions existing at the present time in central

Illinois." That they consider that *G. saubinetii* plays an important part in soil contamination is suggested by the statement (*4, p. 323*) that "In the experiment at Bloomington in 1920 there was a difference of 14.1 bushels in acre yield between corn from good seed and from scutellum-rotted seed where corn followed clover, but in the same field where corn followed badly scabbed spring wheat the difference was 33.1 bushels."

In the Plant Disease Reporter (*9*) the cause of corn root rot is given as *Gibberella saubinetii*.

It is the object of this paper to point out the fact that there is a root disease of corn distinct from seedling blight and its after effects, and that the organisms commonly considered as etiologic factors in corn root rot probably are not concerned in the problem.

SOIL-STERILIZATION EXPERIMENTS

EXPERIMENT 1

For this experiment two lots of soil were collected April 7, 1924, one from a field in corn continuously from 1911 to 1923, and the other from a hardwood forest near by on the same type of soil, which may be considered virgin. The samples were collected and the work done in the spring of 1924. The soils were treated, or not, as follows: (1) Virgin soil untreated; (2) virgin soil steamed at 20 pounds pressure for one hour; (3) corn soil untreated; (4) corn soil to which a mineral fertilizer was added;[2] (5) corn soil steamed at 20 pounds pressure for one hour; (6) corn soil treated with formaldehyde;[3] and (7) corn soil steamed and fertilized.

Uniform plants were not used throughout, seed-borne organisms being eliminated [4] from the plants used in the virgin soil and the soils which were partially sterilized, but they were not eliminated from the plants in the natural corn soil. All series were run in duplicate.

The average dry weights of the roots and tops of the two plants per pot in the two series, one of which was removed on the eightieth day and the other on the ninety-third day, are given in the following tabulation:

	Tops	Roots
	Grams	*Grams*
Virgin soil, untreated	190.5	27.7
Virgin soil, steamed	214.9	13.5
Corn soil, untreated	52	14.1
Corn soil, fertilized	125.7	28.3
Corn soil, steamed	214.2	29.9
Corn soil, formaldehyde	145.3	18.5
Corn soil, steamed and fertilized	203.7	18.1

[2] The chemicals used and the rate per acre were as follows: Acid phosphate, 500 pounds; lime, 200 pounds; KCl, 200 pounds; and nitrate of soda, 200 pounds.

[3] The soil was saturated with a solution of 1 c. c. of 40 per cent formaldehyde to 350 c. c. of water heated to 90° C. The pots were allowed to stand three days and were then emptied and the soil aerated, dried until it worked well, and then sieved and replaced in the pot.

[4] The seeds were treated in water 24 hours, followed by 11 minutes soaking in hot water at 55° C. and then 15 minutes in 1:1000 mercuric chloride solution, and washed three times in sterile water (Sherbakoff's method (7)). They were then planted in sterile sand. When rooted, the plants were removed from the sand, the endosperm and seed coats were removed, and the roots were washed in a weak calcium hypochlorite solution. The endosperm and seed coats were removed from the untreated seeds used in the natural corn soil in the same way, but were replaced with the seedling. The plants were grown in 4-gallon pots, in the bottom of which a small flowerpot was inverted. A glass tube which extended above the surface of the soil was inserted in the hole in the pot and water was introduced through this tube.

The results of this experiment may be summarized as follows:

The roots of 80-day-old plants grown in virgin soil steamed and unsteamed were clean when washed out, showing no lesions; the 93-day-old roots in unsteamed virgin soil showed an occasional small lesion when washed out.

The roots grown in unsteamed, unfertilized, and fertilized corn soil were brown throughout and were very much decayed, while in the same soil steamed they were white and healthy throughout (fig. 1).

The roots from corn soil treated with formaldehyde were clean and white throughout, showing no signs of injury, the temporary root system being still alive and healthy.

The application of fertilizers to the unsteamed corn soil did not decrease the amount of injury to the roots although the root systems in this soil were practically twice as large as those grown in unsteamed unfertilized soil, on the basis of dry weights.

Soil sterilization in the presence of abundant fertility seems to result in the development of a smaller root system (but larger tops) than in unsteamed soil, whether the unsteamed soil is infected with root rot or not.

As the results obtained can not with certainty be attributed to soil infection, but might be explained on the basis of seed infection, these tests will be used only to indicate the importance of corn root rot in the economy of the corn plant. However, the writers believe the results were not due to seed infection, although *Fusarium moniliforme* and possibly other common seed organisms were present in the untreated seed, yet the growth of the seedlings indicated that they were not affected appreciably by these organisms; and the type of injury obtained in the old corn soil has never been observed by the writers to occur in pot work as a result of planting infected seeds, although many root systems have been examined. Subsequent tests reported in this paper show that the typical corn root rot may be produced when fungus-free seedlings are used.

EXPERIMENT 2

A second experiment was planned on much the same basis as experiment 1, using three soils as follows: (1) Virgin soil as in experiment 1—untreated, steamed, and steamed and fertilized; (2) soil from a continuous-corn plot not manured or fertilized; this was used untreated, steamed, with fertilizers, and with fertilizers and steamed; and (3) continuous-corn soil heavily manured each year; this soil was used steamed and untreated.

In this test, treated seed (as in the previous experiment except that in this case heated to 50° instead of 55° C.) only were used in the virgin soil; in the continuous-corn soil, treated and untreated seed were used, respectively, in both steamed and unsteamed soil, in order to determine the relative importance of seed infection and soil infection; while in the manured corn soil treated seed were used in the steamed soils and untreated in the unsteamed soils.[5] All tests were run in duplicate in 4-gallon pots equipped as in experiment 1. Thirty-eight pots were included in the original plan of this experiment, but owing to accidents five of them were eliminated.

[5] Steamed pots were heated in an autoclave for 3½ hours at 18 to 20 pounds pressure.

Examination of the root systems was made after 104 days (Jan. 9, 1925) by removing the ball of dirt as carefully as possible from the jar and working the dirt away from the roots with the fingers and

FIG. 1.—Roots of corn plants grown in soil from a field continously in corn from 1911 to 1923. A, roots from untreated soil; B, roots from soil to which fertilizer was added; and C, roots from the same soil steam-sterilized. The roots in C are clean and white in contrast with the severely injured roots grown in the natural soils. As indicated by the relative size of the root systems, the disease in pots A and B was rather slow in developing

then with water. Without going into all details, the following results were obtained:

Steaming and fertilizing had no appreciable effect, as compared with untreated soil, on the development of plants grown in virgin soil. The root systems in all cases were healthy throughout, and the plants made about equal growth.

No differences could be noted in the development of plants in untreated continuous-corn soil when grown from treated and from untreated seeds. In both cases the root systems were nearly completely decayed when examined.

There was evidence in some of the steamed pots that seed treatment and selection of seedlings had not been entirely effective in eliminating seed-borne organisms, as evidenced by the fact that sometimes the cotyledonary internode and the roots coming from it were found to be rotted. The injury, however, was limited to rootlets in an area not more than 2 inches in extent and was very slight. This is the usual effect of the seed-borne organisms most commonly found in well-cured, good seed corn.

The addition of mineral fertilizers to the unmanured continuous-corn soil increased the size of the plants and the extent of the root systems, in both the steamed and the unsteamed pots. It apparently had no effect whatever on the health of the roots in the unsteamed soil, however, as they were nearly completely destroyed when examined (fig. 2).

High fertility maintained by manuring in the manured continuous-corn soil had a marked effect on increasing the number of healthy roots present on these plants, and also on the size of plants. The plants grown in this soil not steamed were as large as those grown in the same soil steamed and in virgin soil, in spite of the fact that the roots were severely rotted (fig. 2). This was evidently due to the fact that the plant made a vigorous start because of available fertility, so that when the roots began to decay the plant was sufficiently vigorous to replace them as they rotted and so maintain its growth. Numerous healthy roots and rootlets were found in the upper 4 inches of soil, while below that point nearly all the roots were dead. In Figure 2 it will be seen that the plant raised in the steamed soil developed 8 permanent roots, whereas that in the diseased soil developed 17. Evidently the difference is not genetic, as the corn used was a three-times selfed strain of Boone Country White which showed a high degree of uniformity in the field and in the pots. The condition of this plant in diseased soil is typical of plants growing in diseased soil in the field, which, when they have attained nearly maximum growth, have lost nearly all of their root systems by decay but have a very marked ability to partially replace the system after rains. This is undoubtedly the reason why the results of corn root rot other than seedling injury due to infected seeds are often so obscure, even though it may readily be seen by pulling a plant that most of the roots are decayed.

From these experiments and from other tests conducted by the writers it may be definitely stated that death of the temporary root system and of the small laterals and larger roots during the growth of a corn plant is not a normal process, as the individual roots, in the absence of injurious organisms, appear to live at least beyond the tasseling stage. The question whether death of the small roots was due to disease or to a natural process within the plant had been raised on several occasions by colleagues during the progress of this and other work on the roots of crop plants.

EXPERIMENT 3

As some difficulty is at times experienced in growing plants in soil which has been steamed at high pressure for extended periods, it was desired to find the lowest temperature and the shortest period of heating which would eliminate the cause of injury to corn roots. An experiment was therefore planned to determine this point. Soil was obtained April 3, 1925, from a field on the experiment station farm which had grown several successive crops of silage corn. After sieving and thoroughly mixing, the soil was placed in 26 one-gallon glazed jars. Pairs of pots were then treated respectively as follows: Steamed at 14 to 16 pounds pressure for 2 hours and the soil immediately spread in a thin layer to cool and then replaced; treated in

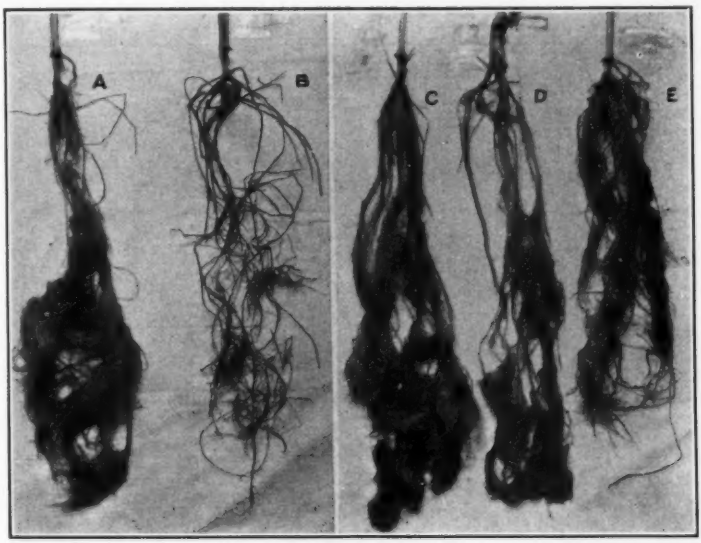

FIG. 2.—A, B, roots of corn plants grown in unfertilized continuous-corn soil to which mineral fertilizers were added for this experiment. The soil in which the plant A grew was steamed, while that in the case of B was not. Fertilization of this soil increased the size of the root systems over those in unfertilized soil, but it did not reduce root-rot injury in the unsteamed fertilized soil. C, D, E, root system of corn plant grown in untreated virgin soil (C), in manured continuous-corn soil steamed (D), and in the same soil unsteamed (E). The roots of C and D are uninjured, but those of E are severely injured, most of the fine laterals being destroyed and the larger roots discolored. More permanent roots developed from the crown of this plant (E) than from the crown of the plant in the steamed soil (D)

the same way as the previous lot but left in the jar after heating; 100° C. for 1 hour; 80° to 85° for 1 hour; 65° to 70° for 1 hour; 65° for 15 minutes after the center of the soil mass had attained this temperature; 40° to 50° for 1 hour; soil air-dried 1 week away from direct sunlight; air-dried for 2 weeks; air-dried for 4 weeks; soil treated with formaldehyde vapor by placing soil on a wire gauze and boiling a solution of 1 part of 40 per cent formaldehyde to 2 parts of water under it for 30 minutes. Two pots were left as checks with no treatment. On April 15, 1925, each pot was set with a single plant of each of two selfed lines of Boone County White corn, one of which

had been self-pollinated five times and the other three times. These plants were completely freed of all seed-borne organisms by a method described in a footnote.[6]

The complete results of this test are not of interest in this paper except as they bear on the problem of soil infection. The roots of all of the plants were examined when 72 days old, with the exception that the two untreated pots and one of the pots steamed under pressure were examined at the end of 47 days. At this time the root systems of the plants grown in the untreated soil were less extensive than those of the plants grown in steamed soil, were considerably discolored, and many of the fine laterals had disappeared. Lesions were present on some of the larger roots. Many of the rootlets had a lemon-yellow cast, although otherwise appearing healthy. The roots of the plant grown in the steamed soil were pure white

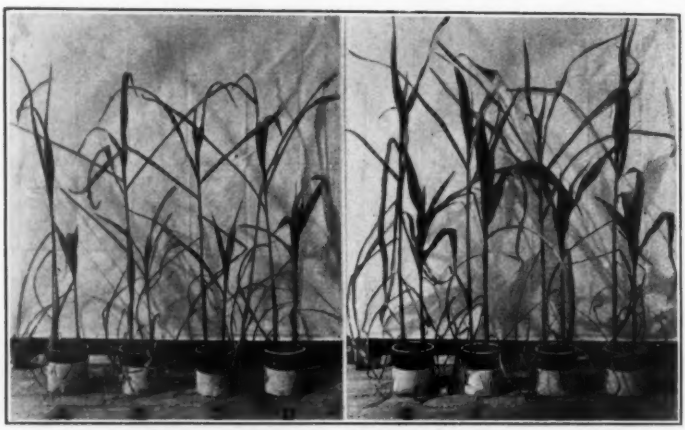

FIG. 3.—Growth of corn plants of two self-pollinated strains growing in continuous-corn soil with various heat treatments. A, soil air-dried 1 week. B, soil air-dried 2 weeks. C, soil treated at 40° to 50° C. for 1 hour. D, soil treated at 50° to 60° for 1 hour. (The plants in A, B, C, and D are comparable in size, with the exception of the small strain in pot D, which seems to show some benefit from the treatment.) E, soil heated to 100° for 1 hour. F, soil heated to 80° to 85° for 1 hour. G, soil heated to 65° to 70° for 1 hour. H, soil heated to 65° for 15 minutes. (The plants in E, F, G, and H are all larger than those in the other four pots. The root systems were white and without injury throughout, in contrast to rotting and lemon-yellow color of rootlets in A, B, C, and D)

throughout, having no rotting rootlets and none of the yellow cast mentioned. At the end of 72 days the remaining plants were of two sizes.

The plants grown in the soil that had been treated at 65° for 15 minutes and at higher temperatures were larger than those in that which had been treated at 50° to 60° or at lower temperatures or air dried (fig. 3). The reason for the difference was evident when the roots were examined. The roots of the larger plants were white and entirely without injury. In some instances the cut end of the internode where the seed was removed was not even discolored but

[6] Briefly, the method consists in treating the seed 20 hours in a mixture of 1 part of CaO to 4 parts of water, followed by 20 minutes in a 1:1,000 mercuric chloride solution. The seeds are then germinated in culture dishes, and when the paired seminals appear they are removed together with the tap root. The seedling is then placed in a tube of nutrient agar where roots are developed from above the seed. When these roots are well developed the plant is removed from the tube, the seed cut off, and the rooted cutting planted. The entire procedure is carried out under aseptic conditions.

had healed over. Sixty-five degrees C. for 15 minutes appeared entirely effective in freeing the soil from organisms injurious to corn roots.

The root systems of plants grown in air-dry soil or soil which had been heated to 50° to 60° C. or below were less extensive, many of the small laterals having been destroyed and some of the larger roots extensively rotted, especially those on the bottom of the pot (fig. 4). The lesions on the roots in the soil that had been heated at 50° to 60° for 1 hour appeared to be more recent and less extensive than in the others of this group, suggesting delayed development of the rot organism due to heating. The injury to all of these root systems was less extensive than in the previous experiment, probably because the period of growth was shorter. Under field conditions the disease appears to act in the same way, not developing

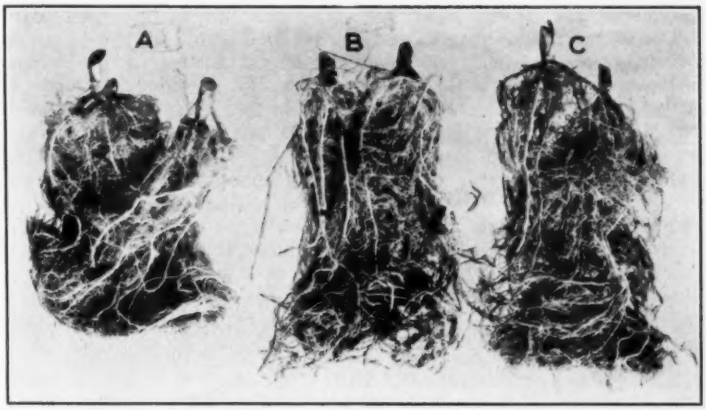

FIG. 4.—The effect of heating continuous-corn soil on the health of the root systems of corn plants. A, soil heated 65° C. for 15 minutes. B, soil heated to 50° to 60° for 1 hour. C, soil air-dried one week. The two latter pairs of plants have many large and small roots which are rotted and dark-colored, especially at the bottom of the pots; the small rootlets of these plants were often lemon-yellow. The roots of A were white and healthy throughout. Heating at 65° for 15 minutes appears to have destroyed the corn root-rot organisms, and the organism which causes the lemon-yellow color of rootlets

seriously until the plants have attained considerable growth, even in soil planted with corn continuously. There appear to be exceptions to this in which in certain areas the disease may become severe early in the life of the plant, resulting in high mortality.

Aside from the differences in rotting in one set of roots and perfectly healthy roots in the others, the two lots of roots (treated above 65° C. and below this temperature) could be separated readily on the basis of the color of the fine rootlets. The healthy roots were all pure white, while many of the rootlets of the diseased root systems were a light lemon-yellow, although appearing healthy otherwise. It is the same color which Jones (*5*) has described as resulting from infection with his mycorrhizal fungus. It should be noted that the roots produced in the air-dried soils had more yellow rootlets and less root rot than the two lots grown in soil treated at 40° to 50° and 50° to 60°, while within these two lots a marked difference could also be noted. The roots from the higher temperature treatment

had markedly fewer yellow roots and more rotting roots (although the rot appeared of more recent origin) than those from the soil treated at the lower temperature. This result suggests that the mycorrhizal fungus (if this is the cause of the yellowing) exerts a slight protective effect against the rot organism, especially in the smaller rootlets.

This experiment has demonstrated that a corn root rot exists which is caused entirely by organisms carried in the soil, and that in untreated soil the disease does not act as a seedling blight but causes its most severe injury later in the life of the plant.

The soil treatment with formaldehyde, although it appeared to have killed all injurious organisms, left the soil in such a toxic condition that replanting had to be repeated several times before a stand was obtained.

In a second test in which soil collected at the same time as that in the preceding test was used, and in which fungus-free corn plants were used, it was determined that in infected soil kept moist at room temperature for 94 days, both the root-rot organism and the mycorrhizal organism were reduced as compared with soil kept air-dry for this period. This relationship held in both series of pots, one set of which was examined 73 days after setting and the other 94 days after. At the first examination, roots developed in soil kept air-dry for 92 days and then exposed to intense sunlight for 14 hours had decidedly fewer yellow rootlets than either those in moist soil or in air-dry soil, while the extent of rotting was slightly less than in air-dry soil. At the time of the second examination, the roots in air-dry and air-dry and sunlight soils were about identical as to yellowing, but roots in the sunlight soil again showed less rot than those in the air-dry soil. Both showed more yellowing and rotting than in the soil kept moist.

SAND-CULTURE STUDIES

In a preliminary experiment started September 9, 1924, sand in 1-gallon glazed jars, to which a nutrient solution [7] was added, was used in place of soil.

Plants from treated [8] and from untreated seed were used. Two pots of treated plants were inoculated with the washings from two culture plates of a corn strain of *Fusarium moniliforme;* two were inoculated each with 30 gm. of roots from corn plants grown in a field which had been planted with corn each year for at least 9 years; two were set with contaminated seedlings, and two with treated seedlings.

The results were as follows: Contaminated seed resulted in injury to a few of the roots in the immediate neighborhood of the seed, the remainder of the roots being clean. The rootlets were clean and white on the plants from treated seed, although some rotting was evident on the temporary roots. *Fusarium moniliforme* destroyed the temporary root system but caused very little other injury than rotting of an occasional small lateral. In the pots to which corn roots were added the roots were nearly completely destroyed (fig. 5).

[7] The nutrient solution consists of KH_2PO_4, 1 gm.; $Ca(NO_3)_2$.3gm.; $MgSO_47H_2O$ 6 gm. in one liter of water.

[8] The seed were treated by the hot-water bichloride method, germinated in steamed sand, and, when well started, removed and the seed cut off leaving the temporary roots intact. Only plants were used which appeared to have fungus-free seeds. Before setting, the plants were immersed in a weak calcium hypochlorite solution. This method is probably not efficient in freeing the plants from seed-borne fungi.

From this test and other observations it may be concluded that *Fusarium moniliforme* plays but little part in the economy of the corn plant; that it is a semiparasitic organism which appears to develop on the plant only to the extent necessary to complete its life cycle of entering the seeds, remaining there over winter, and then

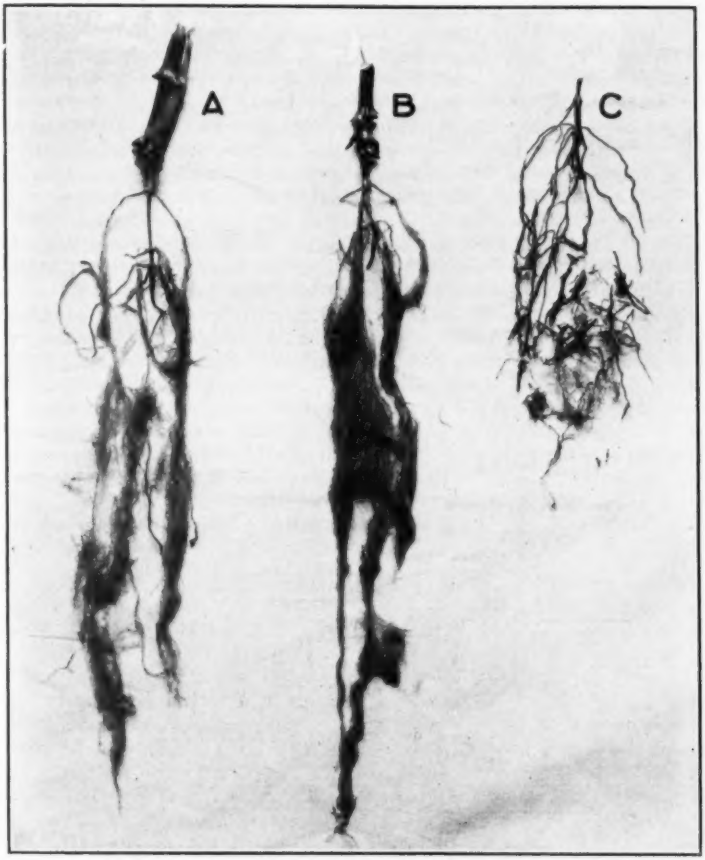

FIG. 5.—Showing effect of rotten corn roots from a continuous corn field on corn plants in sand culture. A, roots inoculated with washings from a culture dish of a pure culture of a corn strain of *Fusarium moniliforme*. B, check; no organisms added. C, sand contaminated with rotten corn roots. Treated seedlings supposedly free from seed-borne fungi were used in each case

developing to a sufficient extent on the roots so that spores will be produced so that it may again gain access to the seed; that it appears to play an important part in the destruction of the corn plant after maturity; and that organisms are present in rotten corn roots capable of causing root rot when introduced into sand cultures.

COMPARATIVE EFFECTS OF GIBBERELLA SAUBINETII AND OTHER ORGANISMS ASSOCIATED WITH CORN ROOT ROTS AND DISEASED CORN ROOTS WHEN USED AS INOCULUM

It is evident from the preceding results that a disease of corn roots may be produced in pots of either soil or sand which is similar to the disease which develops in old corn soil under field conditions, in that the injury is not a seedling blight, but develops to a serious extent after the plants have passed the seedling stage and have started to make rapid growth. Eventually, both in pot work and in the field, practically the entire root system is destroyed. It is believed that this disease is identical with corn root rot as it occurs in the field.

Several pot experiments have been conducted to determine whether this type of root rot can be produced by the organisms which are commonly supposed to cause corn root rot, namely—*Giberella saubinetii*, *Diplodia zeae*, *Fusarium moniliforme*, *Fusarium* spp. The results of these tests may be summed up by saying that *Gibberella saubinetii* acts as a seedling blight, but if the plants recover from the initial injury, they develop in the infected medium without further trouble. Similar results have been obtained, also, with *Fusarium succisae*, which appears to be even more injurious than *G. saubinetii* to corn seedlings. Fusaria of the elegans section, and *F. moniliforme*, both isolated from rotting corn roots, do not cause the severe seedling blight caused by *G. saubinetii* and *F. succisae* but they probably cause injury to a few rootlets. They have very little or no effect on the development of the plant. The results with Diplodia are not conclusive, as there appears to have been an accidental contamination from pots inoculated with diseased roots. The experiments from which these conclusions were drawn will now be described.

EXPERIMENT 1

In this experiment two series of plants were grown, one in soil and the other in sand cultures. The soil used was from the experiment station farm. It had been steamed under pressure several weeks before. The cultures of interest in this paper were: (1) Steamed soil to which was added 10 gm. of sterile rice; (2) steamed soil to which were added 10 rice cultures of *Gibberella saubinetii*;[9] (3) steamed soil plus cultures of a corn strain of *Fusarium moniliforme*; (4) steamed soil plus *F. succisae* cultures; (5) virgin soil to which were added sterile corn roots; and (6) sterile virgin soil to which were added corn roots from a continuous-corn plot where root rot was known to be present.

The plants used were of a three-year selfed line of Boone County White. The seeds for both series were treated by the lime-bichloride method, washed in sterile water, and then were transferred to tubes of nutrient agar for germination. Those which showed no signs of infection when the plant had reached the plug were then transferred, two per pot, to the soil. The plants were set January 31, 1925. In 19 days the plants in all the pots were 12 to 16 inches high. The plants in the soil inoculted with *Fusarium succisae* were at this time about two-thirds the size of the plants in the other pots.

[9] *Fusarium succisae* was isolated from a tobacco root. It was found in test-tube cultures to be very injurious to corn seedlings, and was therefore used for comparison with *Gibberella saubinetii*, although it is probably of no significance as a corn pathogene. The *G. saubinetii* culture used was from tobacco roots. Both these organisms were identified by C. D. Sherbakoff.

Both these plants and the plants inoculated with *Gibberella saubinetii* had wilted slightly every sunny day until this time, when they both appeared to have developed a sufficiently healthy root system to supply moisture. The others had not wilted. The plants were removed at the end of 40 days. The air-dry weights of the roots and tops were as follows: Check, 48 gm.; *G. saubinetii*, 42.5 gm.; *F. moniliforme*, 41 gm.; *F. succsiae*, 29 gm.; virgin soil and steamed roots, 44.5 gm.; virgin soil and diseased roots, 32.5 gm.

The original roots of the plants inoculated with *Fusarium succisae* were nearly destroyed, as were some of the first permanent roots which developed. The later roots were so nearly entirely healthy that it might be said that the plant was recovering from the initial injury. The plants inoculated with *Gibberella saubinetii* were slightly smaller than the checks and showed but slight injury to the roots. They had apparently been injured in their early development, as evidenced by the daily wilting, but had entirely recovered. The plants inoculated with *F. moniliforme* showed but an occasional injured rootlet. The roots of the check plant were healthy throughout. The roots in virgin soil with sterile corn roots added were healthy throughout, while those in virgin soil plus infected roots showed extensive injury to the laterals, many of them being completely rotted. It appeared that in but a short time the remainder would have been destroyed. In these plants the injury was evidently increasing, whereas the plants inoculated with *G. saubinetii* and *F. succisae* appeared to be recovering.

The sand-culture series of this experiment practically duplicated the soil series, except that steamed sand to which a nutrient solution was added was used in place of the soil. Inoculations were made in the same manner. The rates of growth of the plants treated in the various ways were similar to those growing in soil. The plants inoculated with *Fusarium succisae* and *Gibberella saubinetii* wilted during midday on sunny days. The plants were removed from the pots on the 25th day and the sand washed from the roots. The green weights of the plants at this time were as follows: Check, 35.1 gm.; *G. saubenetii*, 9.7 gm.; *F. moniliforme*, 29 gm.; *F. succisae*, 5.7 gm.; diseased corn roots from field mixed throughout sand, 23.3 gm.; diseased corn roots from a previous experiment placed in lower half of pot, 26.4 gm.; and same planted with an open-pollinated Boone County White plant, 60.5 gm. The roots of the check to which 10 gm. of sterile rice were added were clean throughout, except for a very few dead rootlets in the vicinity of the seed. The roots of the plants inoculated with *F. succisae* were badly rotted. The fungus was growing quite profusely around the crown of the plants. Lesions were scattered over the root system of the plant inoculated with *G. saubinetii*, but the majority of the rootlets were healthy. The injury hardly seemed sufficient to account for the slow growth of this plant, although probably more injury was caused in the seedling stage than was apparent at the time of inspection (fig. 6). The roots of the plant inoculated with *F. moniliforme* were but slightly injured, an occasional rotted rootlet being found. The three remaining pots were inoculated with diseased root material. The first, in which rotted roots collected January 17 from the field were mixed throughout the pot, had developed an extensive root system, but it was nearly completely dead and rotted when examined. In the

other two, diseased roots from a previous experiment, in which corn was grown in infected field soil, were used. The roots were mixed only in the bottom half of the pot. One of these pots was set with an open-pollinated seedling. This plant was nearly twice as large as any of the selfed plants, indicating that its early growth had been rapid and uninfluenced by the inoculum in the bottom of the pot. At the time of removal, although the sand was moist, the leaves were curled and wilted. The roots at the bottom of the pot were extensively rotted, while the rot was spreading more slowly in the rootlets higher up in the pot. The injury evidently was increasing with the age of the plant. In the other pot where the inoculum was placed in the bottom half of the pot, the roots were extensively injured in the lower half with an occasional rotted root higher up. In this series, although *G. saubinetii* and *F. succisae* both caused injury to the plants in their seedling stages, the plants in both cases appeared to recover to some extent, the later roots appearing healthy; whereas plants inoculated with diseased root material made a normal growth in the seedling stage, but developed an increasing amount of injury as the plants grew older. The two diseases can not be considered identical, the former being a seedling blight and the latter a disease of the developing plant.

EXPERIMENT 2

In this experiment 1-gallon glazed jars of steamed sand (18 pounds pressure for 2 hours) were used, to which nutrients [10] were added.

The inoculum consisted of 30 grams per pot of finely cut and washed silage sterilized in 15-gram lots in culture dishes at 15 pounds pressure for 15 minutes. The dishes of silage were then inoculated respectively with pure cultures of *Gibberella saubinetii*, *Fusarium succisae*, and *F. moniliforme*. They were then incubated at 26° C. for 4 days, at the end of which time the silage was overgrown profusely with the organisms, and then added to their respective pots of sand. All were run in triplicate except *F. moniliforme*, which was used in a single pot. Thirty grams of diseased roots, collected from the field February 27, 1925, and cut into small pieces, was added to each of three pots, about a gram of the root material being put in direct contact with the roots of the plant in each case when it was set. Thirty grams of sterile washed silage was added to each of the three checks.

The plants used were a five-times selfed strain of Boone County White, prepared by the limewater-bichloride method, as in the previous experiment, and germinated in tubes of agar. Only apparently fungus-free seedlings were used. The plants were removed from the tubes with the agar adhering and set with the first node just below the surface of the sand. The plants were set March 6, 1925. Ten days later no differences could be noticed.

On March 30 the plants inoculated with diseased roots presented the best appearance; while the checks to which sterile silage had been added were next, but noticeably smaller. Two plants in one pot inoculated with *Fusarium succisae* were practically dead (seedling

[10] Ten grams of finely powdered C. P. $Ca_3(PO_4)_2$ was added at the time the pots were inoculated. After the plants were set, 20 c. c. of a nutrient solution consisting of 10 gm. of KNO_3 and 25 gm. of $MgSO_4 \cdot 7H_2O$ per 1,000 c. c. of water, was added to each pot; and also 10 c. c. of Scales' solution (25 gm. in 1,000 c. c.) was added.

blight). Two plants in one of the *Gibberella saubinetii* pots also appeared very sickly. The other plants inoculated with these organisms were all about the size of the checks, or nearly so. On April 3 the two plants in the above-mentioned *F. succisae* pot were

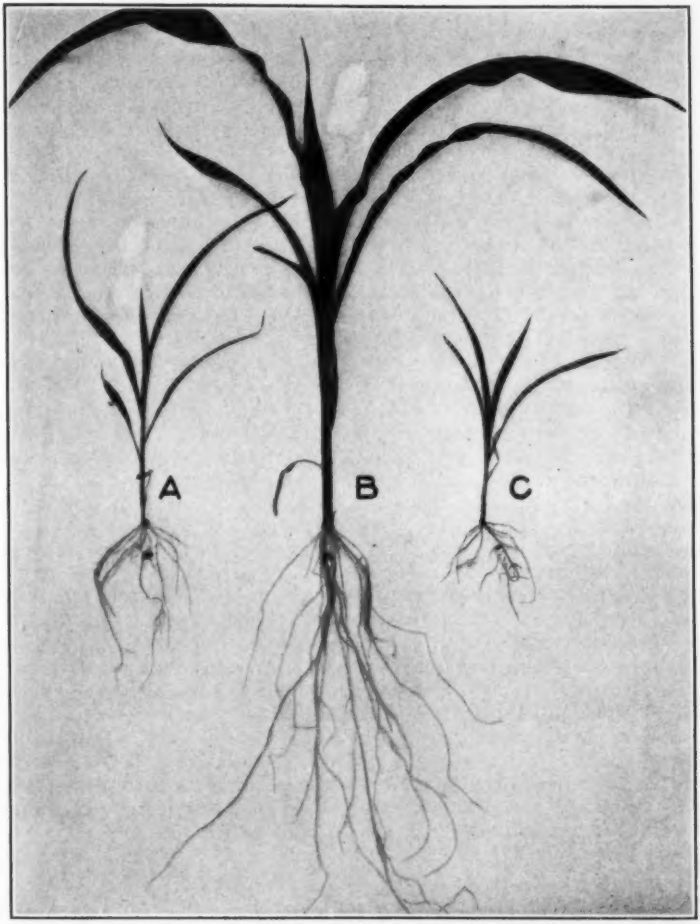

FIG. 6.—The effect of *Gibberella saubinetii* and *Fusarium succisae* on 25-day-old corn plants grown in sand cultures. A, sand, inoculated with rice cultures of *G. saubinetii*. The roots are for the most part quite healthy, although considerable injury to the rootlets probably occurred in the seedling stage. B, check; sterile rice was added. C, rice cultures of *F. succisae* added to the sand. The roots show extensive injury, although the plant appeared to be recovering

removed, as one was dead and the other nearly so. Both had developed fairly extensive root systems with a poor development of laterals, and lesions were scattered here and there on the larger roots. The death of these plants did not appear to be the result of root injury. The internode of the epicotyl and temporary roots

of both plants were rotted, and the rot had spread into the first nodes, killing the plants. On April 9 the plants were removed from one series of pots, with the exception of the *F. succisae* plants, which had already been removed. The *G. saubinetii* pot containing the two small plants was selected. The highest leaves of these plants reached 14 inches. Most of the roots were dead, on one plant only a couple of recent ones being still alive. The first internode of this plant was rotted, the injury probably extending into the first node. The second plant was somewhat larger than the first and was beginning to recover from seedling injury. The original roots and the laterals on the permanent roots were discolored, but a set of healthy roots was developing from the second node.

The plants inoculated with diseased roots were taller than the others of the series (20 inches), but were not so stocky and appeared to wilt more readily. The root systems of the plants removed were extensive and considerably injured but not so much as those inoculated with *Gibberella saubinetii*. Many of the small rootlets were yellow, although not decayed, and when sectioned were found to be penetrated by a fungus answering closely the description of Jones's mycorrhizal fungus (*5*).

The checks were 17 inches high when removed, a few rootlets were found to be discolored, and two larger roots had lesions. Otherwise these roots were healthy. On April 16 all of the series were going ahead of the plants infected with diseased roots. The latter were light-green and spindly, with long narrow leaves, while the others were dark green and had shorter, broader leaves.

On May 22 but little difference could be noted in the size of the plants, with the exception of those inoculated with corn roots, which were smaller (fig. 7). On May 25 the remaining plants were removed for observation of the roots.

The root systems of all plants, except those inoculated with corn roots, were about the same size and very extensive (fig. 8). In the bottom of one of the *Fusarium succisae* pots, and also in the remaining *Gibberella saubinetii* pot, a considerable number of rotting roots were present. An occasional lesion was present in the checks. The roots inoculated with *F. moniliforme* were as large as those of the check, and except for an occasional lesion they were healthy throughout. The roots of the plant inoculated with root material were nearly dead (fig. 8). The contrast in the seedling-blight injury caused by *G. saubinetii* and *F. succisae*, and root rot caused by adding infected roots, is again brought out in this test.

EXPERIMENT 3

In this experiment one-half gallon glazed jars of river sand were used, to which nutrients [11] were added. The inoculum added was, in all cases, root material collected April 13, 1925. It was brought to the laboratory, washed and ground in a food chopper, weighed into 30-gram lots, sterilized (when sterile roots were necessary) in culture dishes, and 15 c. c. of 5 per cent potato-dextrose solution added to each portion. Three lots were inoculated with *Gibberella saubinetti*, and three with a composite of five cultures of Fusaria of the elegans section isolated from corn roots. The cultures of root material were

[11] Seven grams C. P. $Ca_3(PO_4)_2$ thoroughly mixed with the sand, then 10 c. c. of the following nutrient solution was added to each pot: 100 gm. KNO_3 + 25 gm. $MgSO_4 7H2O$ per 1,000 c. c. water.

inoculated by transferring some of the mycelium of the organisms used to the tube of decoction and pouring this over the root material. The plates, including the unsterile roots plus decoction, were incubated for three days and then added to the pots of sand. Fungus-free plants (see footnote 6, p. 460) of a Boone County White strain

FIG. 7.—Eighty-day growth of corn plants in sand cultures as influenced by inoculation with *Gibberella saubinetii* (A), with rotted corn roots (B), and with sterile washed silage (C). The plants inoculated with corn roots made more rapid early growth than the others, but were soon overtaken and outgrown. If those inoculated with *G. saubinetii* pass the seedling stage without injury, they make normal growth. Seedling blight may, however, destroy them

selfed five times were then set, using two plants per pot, on April 16. On May 4 the plants in the pots inoculated with root material were severely injured. At this time plants in the three check pots appeared slightly poorer if anything than those inoculated with *G. saubinetii* and the elegans organisms, although the difference was not great. These

plants were all approximately twice the size of the root-inoculated plants. The early injury to the plants inoculated with root material in this case, as compared with the other experiments where injury was always delayed, may have been due to the fact that the causal organism was activated by incubating three days with a nutrient solution and therefore was capable of attacking the roots immediately when the plants were set. On June 12 all plants inoculated with infected corn roots were dead. On June 26 (53 days) the plants were removed from the pots and the sand washed out of the roots. Photographic records were made and the plants were air-dried. The average air-dry weights of all plants, except those inoculated with corn roots, were as follows: *G. saubinetii*, 26.3 gm.; *Fusarium spp.*, 16.5 gm.; checks, 25.4 gm.

The roots of the check plants were healthy throughout in the three series (fig. 9). The roots in two of the pots inoculated with

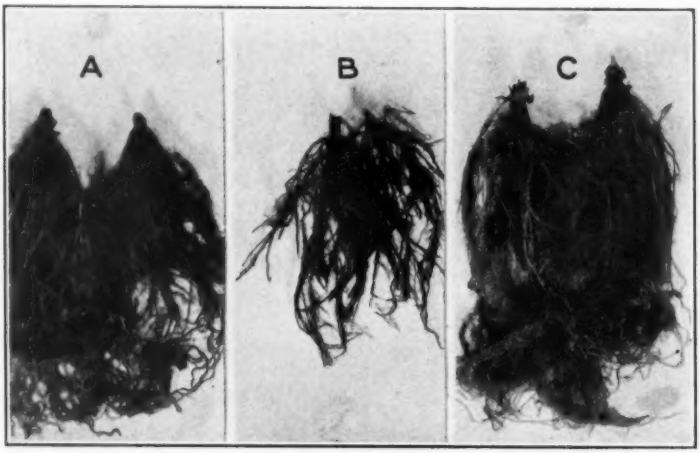

FIG. 8.—Roots of the plants A, B, and C shown in Figure 7. A (inoculated with *Gibberella saubinetti*), a few roots rotting on the bottom of the pot. B (inoculated with rotted corn roots), the roots being completely rotted. C (check), the roots appeared healthy and white throughout

Gibberella saubinetii were practically as good as the check; while in the third the roots were very severely injured, having an appearance typical of the true corn root rot of the other experiments. The roots in two pots inoculated with *Fusarium spp.* were for the most part normal, although some of the early roots had evidently been injured, the third was in exactly the same condition as the *G. saubinetti* plant mentioned above. The roots were nearly completely rotted. As only one set of plants in both the *Fusarium spp.* series and the *G. saubinetii* series showed this condition, the writers assume that the infection was accidental from one of the root-inoculated pots. There has been evidence in other experiments that accidental contamination has occurred from the root-inoculated pots to others.

An experiment in which *Diplodia zeae* infected roots collected in the summer from diseased corn plants in the field, and a composite of 42 distinct cultures of Fusaria, most of which were of the elegans section, were used as inoculum was not entirely satisfactory. The

root-inoculated plants were extensively injured. Those inoculated with *Fusarium spp.* were injured on the bottom of the pot, due in part to decomposition of organic matter which left a black sludge with a distinct odor of sewage. The roots above this area, except for an occasional rotted rootlet, were healthy. The roots inoculated with *D. zeae* had grown extensively, but when removed they were found to be severely injured in both pots. Some unauthorized person had watered the plants during the progress of the experiment with a hose and caused considerable splashing, so it can not be said whether the injury was due to *D. zeae* or to accidental contamination. At least it may be said that *Gibberella saubinetii* and the Fusaria of the elegans section commonly isolated from corn roots are unable to cause corn root rot similar to that caused by rotted corn roots from a continuous-corn field.

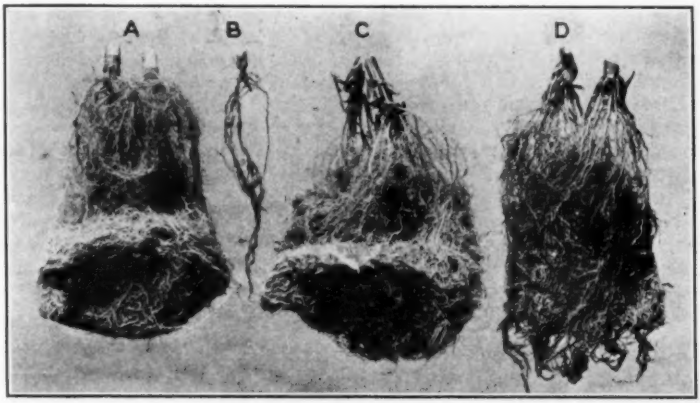

FIG. 9.—The effect of inoculation with rotted sterile corn roots (A), with rotted roots incubated with a nutrient solution for three days (B), and with rotted sterile roots plus a nutrient solution inoculated with *Gibberella saubinetii* (C). D was inoculated in the same way as C. It made rapid normal growth for a time, but gradually fell behind C. When its roots were removed D was found to have what was apparently a typical case of root rot, due probably to accidental infection by splashing from one of the root-inoculated pots. Two pots of plants in this experiment inoculated with a composite of five seemingly different cultures of elegans Fusaria from corn roots looked like C, while a third appeared identical with D and evidently had been infected accidentally

RELATIONSHIP BETWEEN SOIL TEXTURE AND ROOT ROT IN POT CULTURES

In the pot experiments just reported observations were made on the distribution and spread of root rot in the various pot cultures which may throw some light on the cause of the disease. Wherever possible the complete ball of dirt or sand was removed from the jar in order to observe the roots concentrated against the inside of the pot. Rot was nearly always more severe in the bottom of the pot in the early stages of its development, but would gradually spread throughout the entire root system. In one case of evident accidental infection a streak of rotting roots was observed extending down one side of the pot, the rot evidently having followed the downflow of water. Development of rot was always more rapid in sand cultures, in which waterflow is quite rapid and where watering must be done at more frequent intervals than in the less porous soils; in fact, this

difference was so striking that an attempt was made to find a soil-sand combination in which the rot would develop about as rapidly as in sand, but would be furnished with sufficient nutrients from the soil to maintain the plant without the addition of a nutrient solution. The soil and sand combinations used were virgin soil and river sand sterilized, half virgin soil and half sand, and one-quarter virgin soil and three-quarters sand. To each of the foregoing were added rotted corn roots from a continuous-corn plot. Virgin soil plus 30 gm. of sterile roots and sand plus 30 gm. of sterile roots served as checks.

When the roots were finally examined the injury was found to be in direct proportion to the amount of sand present, the roots in soil being injured comparatively slightly, while those in sand were nearly completely destroyed. The plants were grown in 1-liter beakers so that root development and injury could be watched throughout the growth of the plant. During the progress of the experiment, when the plants were about half grown, one of the checks (sand plus sterile roots) became accidentally contaminated, evidently from one of the inoculated pots. The rot spread rapidly and when the plant was removed its roots were found to be nearly destroyed.

It is interesting to note that yellowing of the mycorrhizal type was produced both by virgin soil and by the addition of rotted roots to sterile sand, but was entirely absent in the sterile-sand-plus-sterile-roots plants.

The fact that the disease spread so much more rapidly where the flow of water was uninterrupted, suggested that the cause of the disease was probably one of the fungi which produce swarm spores or at least depended upon some free water for dissemination.

ISOLATION AND MICROSCOPIC STUDIES

Isolations have been made from some of the rapidly rotting roots from pot cultures and from plants grown in the field but have yielded usually either Fusaria or a fungus apparently identical with an organism commonly isolated from tobacco roots which was tentatively-identified by C. L. Shear as a species of Hendersonia or as *Stagonospora arundinis*. *Fusarium moniliforme* was quite often isolated, but much more commonly species belonging in the section elegans of this genus. Tests of these organisms on corn seedlings growing in nutrient agar indicate that whereas they may cause injury under these conditions, yet the injury is of a different type from the true rot, being limited to very small laterals and to a small area of the larger roots. These tests and the pot tests reported above indicate that these organisms are probably not concerned in the problem other than as saprophytes or weak parasites which follow closely the real cause of the trouble. *Gibberella saubinetii* and *Diplodia zeae* were never isolated in these studies; and in approximately 150 separate culture plates, in each of which about five pieces of rotting roots of tobacco from several fields were planted, *G. saubinetii* was isolated but once.[12] Searches made in continuous-corn fields on the experiment station farm for the past several years by the senior author for the perfect stage of *G. saubinetii* have resulted largely in failure, especially where the field lies to the windward of all wheat fields. These results suggest strongly that *G. saubinetii* is not a common corn

[12] MILLER, P. W. [Unpublished master's thesis, Univ. Ky., Lexington, 1923.]

pathogene except as it attacks developing ears of corn, and seedlings as a result of planting seed from such infected ears, and as it attacks matured corn stalks which have been grown in the vicinity of wheat or other small grains. It does not appear to be a common soil organism, except as it is turned under on infected plant parts. It appears to have specialized largely as an above-ground organism.

Free-hand sections of the rotting edge of roots from pot cultures (experiment 3, p. 16) revealed the presence of a large-diameter, usually nonseptate fungus which is evidently a phycomycete. This has appeared consistently at the edge of the rotting tissue in the type of root injury resulting from the addition of rotted roots (fig. 10, A). Behind this zone in the rotted tissue are found osopores, often in large numbers (fig. 10, B, C). Twenty which were measured ranged from 18.8 microns to 27.0 (averaging 23.2) in diameter, including the walls.

A review of the literature on this type of root injury revealed the striking similarity between the sugar-cane root rot described by Carpenter (*1*) and the root rot of corn produced in either sand or soil pot cultures by the use of either diseased soil or diseased roots. (See Carpenter's plates 1 and 3.) The hyphae and the oospores in diseased roots of corn are strikingly similar in appearance to those pictured by Carpenter, and the similarity suggests a relationship between the two fungi. In a later paper Carpenter (*2*) showed the morphological similarity of his Pythiumlike fungus to *Pythium Butleri* Subramaniam and *Rheosporangium Aphanodermatus* Edson. Subramaniam, as quoted by Carpenter, found his organism to be parasitic on *Nicotiana tabacum*, *Zingiber officinale*, and *Carica papaya*. In the present studies the writers have found that rotted corn roots produced a rot of tobacco similar in character to the disease produced on corn roots in pot cultures. Although it is too early to draw definite conclusions in regard to the actual cause of corn root rot, there is a strong suggestion that the Hawaiian root troubles and those of corn in the United States may be very similar in nature. The fact that the writers have not obtained a Pythiumlike organism in isolations from rotting roots is not surprising, as Carpenter had considerable difficulty in isolating the organism until a favorable medium was discovered.[13] In 1919 Clinton (*3, p. 428–430*) obtained plants of corn severely affected with root rot, and found, in the pith of the stubble in the vicinity of the nodes, the oospores of a fungus which he identified as *Phytopthora cactorum*, but he was not certain whether it was a Pythium or a Phytophthora. The oospore measurements and appearance of the spores correspond closely with those illustrated in this paper.

CONCLUSIONS

A series of experiments have been conducted using both soil and sand in an attempt to differentiate between the types of injury caused by the more or less commonly accepted corn root rot organisms. It has been determined that through the use of soil from a continuous-

[13] Since the completion of this work, one of the writers (Karraker) was informed by James Dickson, at the November, 1925, meeting of the American Society of Agronomy in Chicago, that a Pythiumlike fungus had been isolated from corn roots in his laboratory; and more recently still, the program of the seventeenth annual meeting of the American Phytopathological Society announces a paper by Helen Johann, J. R. Holbert, and James G. Dickson on "A Pythium seedling blight and root rot of dent corn."

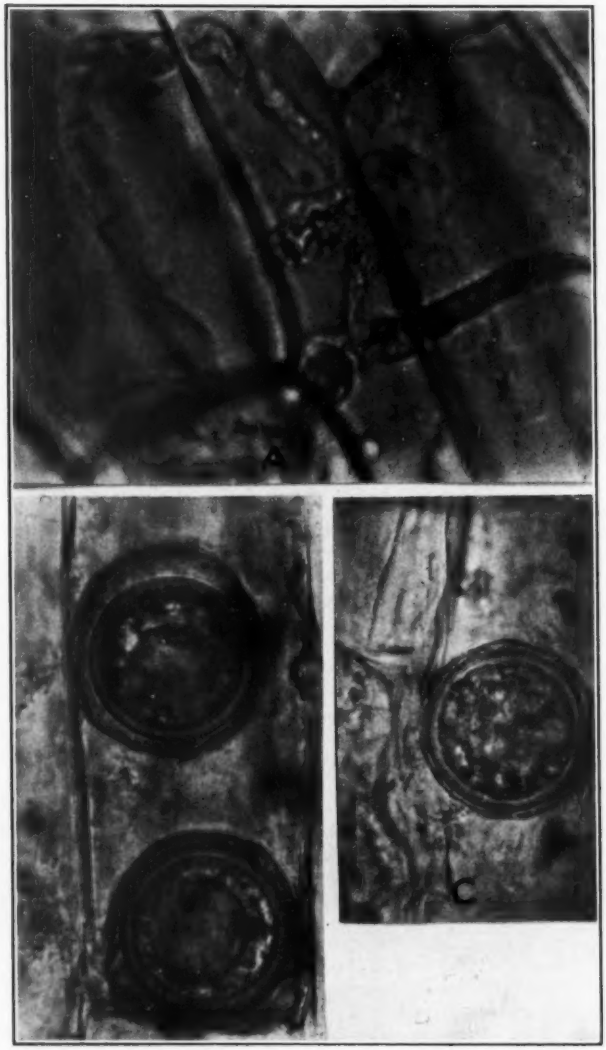

FIG. 10.—A. Pythiumlike fungus near the border of rotting and healthy tissue of a corn rootlet grown in sand culture to which dead corn roots from a continuous-corn field were added. This type of organism has been found consistently in the rotting rootlets examined. Penetration of the host cell wall may be seen in two places. Freehand, × 1000. B, C, Pythiumlike oospores consistently present in the rotting rootlets examined in the decayed zone immediately behind the type of fungus shown in A. These spores are usually abundant in rotted tissue in sand cultures. B, free-hand section; and C, crushed rootlet × 1000

corn plot, in the absence of seed-borne organisms, a severe rotting of corn roots may be produced. The addition of rotten roots collected from a continuous-corn field in the fall, midwinter, and spring, and added to sand cultures in which fungus-free corn plants were grown, resulted in a severe root rot. Rotting was found to progress more rapidly in sand where there was a free movement of water than in soil. The disease did not act as a seedling blight in pot cultures unless the causal organism was previously activated by incubation of the rotted roots with a nutrient solution.

Both *Gibberella saubinettii* and *Fusarium succisae* have been found to cause seedling blight, but if plants escape seedling injury, they appear to make as rapid growth as check plants. In more than 100 isolations made from rotting corn roots from plants growing in the field, in pot cultures of diseased soil, or in sand cultures inoculated with rotten corn roots, these organisms have not been obtained in a single instance. *Diplodia zeae* has not been obtained in these isolation studies. *F. moniliforme* and species of Fusarium of the elegans section are commonly isolated from rotting corn roots. *F. moniliforme* appears to rot only an occasional root in sand cultures, and a composite of 42 cultures of Fusaria from corn roots, most of which were of the elegans section, caused but slight injury. Apparently they are concerned in the problem only as secondary invading organisms.

Free-hand sections of rotting corn roots grown in infected soil, or in sand cultures inoculated with rotten corn roots, have consistently revealed a large-diameter, generally nonseptate organism, and numerous oospores of the Pythium type. The type of injury produced in soil and sand cultures is remarkably similar to the injury (pictured by Carpenter) to Lahaina cane grown in sick soil and caused by a Pythiumlike fungus. The suggestion is made that corn root rot, other than seedling blights known to be caused by certain seed-borne organisms, is caused by a fungus similar to the fungus causing the cane root rot.

Rotted corn roots added to sand cultures of tobacco are capable of causing a type of injury which appears identical with the so-called brown root rot of tobacco in the field.

The roots of corn plants grown in continuous-corn soil, in virgin forest soil, or in sand to which rotted corn roots from the field have been added, develop lemon-yellow discolorations; and on sectioning the roots are found to be penetrated by a fungus similar to the mycorrhizal fungus described by Jones. This organism and the corn root-rot organism are both destroyed by maintaining the soil at 65° C. for 15 minutes.

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A BLOSSOM AND SPUR BLIGHT OF PEAR CAUSED BY A STRAIN OF BOTRYTIS CINEREA PERS.[1]

By S. M. ZELLER
Plant Pathologist, Oregon Agricultural Experiment Station

INTRODUCTION

As far as the writer is aware, this paper is the first report in the literature of Botrytis having been found to be the cause of a blossom blight of pear, *Pyrus communis*. This blight of *Pyrus communis*, caused by a strain of *Botrytis cinerea* Pers., was first reported by the writer [2] in 1920, at that time as a disease of the spur. In April and May of that year specimens of blighted spurs were sent to the Oregon Agricultural Experiment Station from two localities in Douglas County, Oreg. In one orchard in one of those localities from 10 to 15 per cent of the fruiting spurs of some Anjou pear trees were infected, and somewhat less infection was found in Winter Nelis

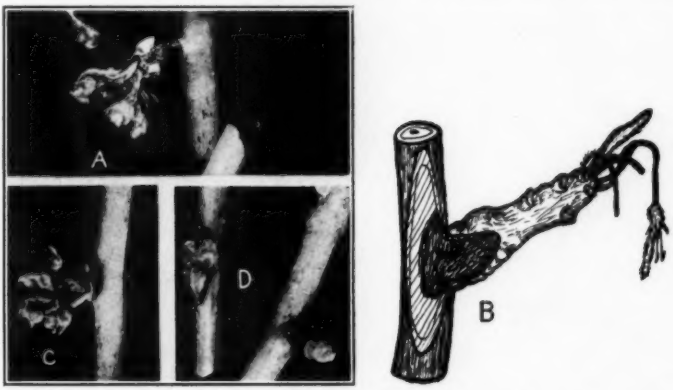

FIG. 1.—A, Diseased spur of Anjou pear that was attacked by Botrytis "in the pink" stage of the buds. When this photograph was taken the invasion had extended into the parent branch; B, diagrammatic illustration to show how far the mycelial invasion and discoloration progressed into the parent branch in one instance; C, photograph of a diseased spur taken the same spring that infection took place. Note the cankerous condition at the base of the spur; D, photograph showing canker at the juncture of the spur with the parent branch

and Comice trees. Since 1920 the disease has been found mainly on Anjou stock, but Bartlett, Nelis, Comice, and Bosc have been slightly affected in the order mentioned.

DESCRIPTION OF THE DISEASE

Infection takes place through the flower parts. The first visible evidence of the disease is the wilting of one or more flowers or flower

[1] Received for publication Jan. 20, 1926; issued September, 1926.
[2] ZELLER, S. M. A SPUR BLIGHT OF PEAR CAUSED BY BOTRYTIS. (Abstract) Phytopathology 11: 105. 921.

buds of a spur. If only one flower is affected, the mycelium makes its way down the flower pedicel in one or two days. When the infection reaches the base of the pedicel it spreads to the bases of other flower pedicels, and the flowers which these pedicels support quickly wilt, and the whole spur has a diseased appearance (fig. 1, A). As is always true in the case of pear tissues, the affected parts quickly darken. Infection and discoloration continue rapidly down the spur and advance for some distance into the parent branch (fig. 1, B). This whole invasion takes place within two to four days, so there may be many thoroughly diseased spurs before the petals fall from healthy flowers. Fortunately, infection takes place at a time of vigorous growth of the parent branch. The vigorously growing pericycle producing a cork cambium about the diseased area at the base of the spur serves as an expeditious check against the further advance

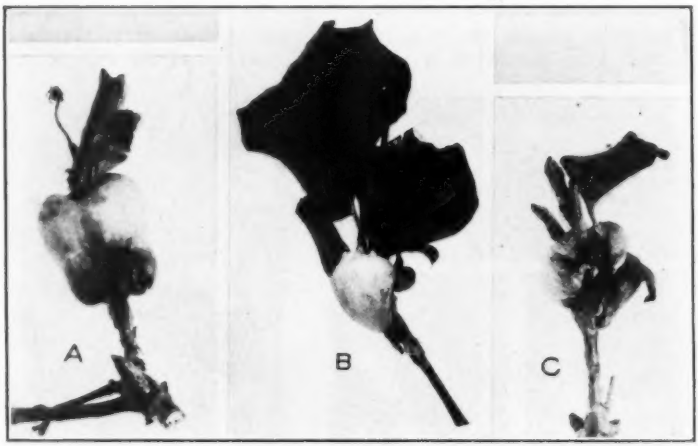

FIG. 2.—Photographs of the vegetative fruits produced just above cankers that are caused by Botrytis on Anjou stems or petioles. In A, the canker has forced the fruit away from the cortex of the stem. In B and C the fruit is an enlargement of the petioles

of the mycelium. As soon as the spread of the mycelium is checked the base of the diseased spur becomes cankerous in appearance, owing to the heaving and splitting of the margins of the dead bark when callus begins to form (fig. 1, C). In a short time the flower parts fall away, and in late spring and early summer the diseased spurs appear, as illustrated in Figure 1, D. If the spurs are taken during these stages of the disease, the causal organism may be isolated from the diseased tissues.

Although such cases are seldom found, infection may occur after the fruit has set. In such a case infection starts through the sepals, but the invasion progresses more slowly than described above. The invasion slowly spreads in the cortex of the immature fruit, which usually drops before it is 8 to 10 mm. in diameter. In cases of fruit infection the fungus seldom gets beyond the suberized layer of the normal abscission layer at the base of the pedicel. When the in-

vasion does exceed this limit the small area of diseased tissues below the abscission layer is limited, being occluded from the vigorously growing healthy tissues by a rapidly formed phellogen layer. These single late-infected fruits or flowers dry and fall away late in the spring or early summer. Collateral products of the small cankers on the parent branch are the vegetatively produced false fruits. Parthenocarpy in Anjou pears when floral parts are present has been illustrated and discussed by Kraus and Kraybill.[3] When Botrytis canker occurs in such a position on a small twig as to cut off the downward translocation of food substances from leaves above, these vegetative pears may be found (as illustrated in fig. 2, A, B, C) immediately above the canker as a fleshy enlargement of the outer cortex of the stem or of the petioles of the leaves furnishing the nourishment

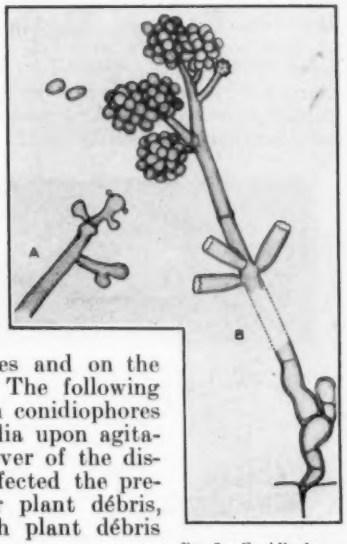

FIG. 3.—Conidiophores and spores of the strain of *Botrytis cinerea* Pers. which causes blossom and spur blight of pear. A, mature conidiophore. × 280. B, immature conidiophore, with spores beginning to form

DISSEMINATION OF THE DISEASE

The Botrytis organism remains dormant during the summer in the diseased spurs and cankers. In the fall, after the rains begin, sclerotia are formed in the crevices and on the surfaces of the diseased tissues. The following spring the sclerotia are covered with conidiophores (fig. 3), which shed clouds of conidia upon agitation of the spurs. Since the hold over of the disease is in the spurs which were infected the preceding season, as well as in other plant débris, control by the elimination of such plant débris would be hopeless.

PROOF OF PATHOGENICITY

The pathogenicity of this strain of *Botrytis cinerea* has been established by the constant association it has with the type of injury described above, and by inoculations made with conidia from pure cultures. The disease has been collected many times in the Umpqua Valley in Oregon, and has been sent to the Oregon Agricultural Experiment Station from several localities in that district and also from the Grants Pass and Medford districts of the Rogue River Valley. In 1923 it was reported from Antelope Valley and similar localities in California.[4] The causal organism has been isolated from samples of all of the cases reported in Oregon.

Inoculations were made in three different ways: (1) Spores were dusted on the flower parts; (2) spores were sprayed on in a water suspension; and (3) a water suspension of spores was injected into

[3] KRAUS, E. J., and KRAYBILL, H. R. VEGETATION AND REPRODUCTION WITH SPECIAL REFERENCE TO THE TOMATO. Oreg. Agr. Expt. Sta. Bul. 149, 90 p., illus. 1918.
[4] SMITH, E. H. MINOR APPLE AND PEAR DISEASES. Calif. Agr. Expt. Sta. Rpt. 1922/23: 185. 1923.

the bark of spurs with a hypodermic needle. In the cases where the latter method was used the spore dilutions in sterile water were injected into 20 spurs each of Anjou and Bartlett pears on March 23, 1921. Nineteen (or 95 per cent) of the Anjou spurs were killed in 6 to 12 days, while 16 (or 80 per cent) of the Bartlett spurs were withered in the same period. When spores from a pure culture which was obtained from wilted Anjou flowers were dusted on Anjou blossoms in the greenhouse, the blossoms being in all stages from "in the pink" to full bloom, 20 spurs showed wilted blossoms in 4 days while 20 spurs which were not dusted with spores were normal. Infection was accomplished as successfully when the spores were sprayed on flowers as when they were dusted on. The dusting inoculations were repeated under field conditions. Out of 30 Anjou spurs inoculated with dry conida 26 showed one or more blossoms wilted in five days. The relation of humidity and temperature to infection has not been studied.

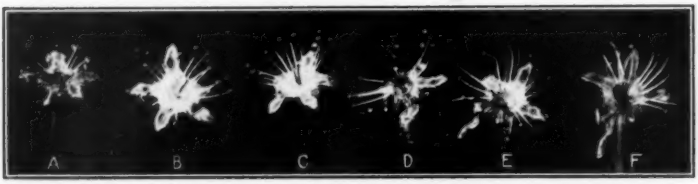

FIG. 4.—Pear flowers, with the petals plucked away so as to show the various courts of infection. A, about half of the flower infected; B, one style infected; C, two styles infected; D, all of the styles infected and the infection is advancing into the calyx cup from two sides; E, Infection from a petal has nearly reached the pistils. Two styles, seen to be partially discolored, were infected later; F, petal infection has extended into the calyx

INCIPIENCY OF INFECTION

Ward,[5] in his classic study of a lily disease caused by *Botrytis cinerea*, demonstrated that infection was possible through extracellular enzyme activity which digested the superficial walls of the host tissues. On the other hand, Blackman and Welsford[6] observed that the germ tube of *B. cinerea* on the surface of the leaf of broad bean (*Vicia faba*) excreted a mucilaginous sheath by which the tube was firmly cemented to the surface. A depression in the epidermal wall below the germ tube suggested that considerable pressure was exerted on the substratum. Actual penetration of cuticle was by means of a peglike growth that was developed from the underside of the germ tube which was in close contact with the cuticle, and thus penetration could occur without there being an appressorium. No influence of enzyme action on the cuticular walls could be detected, but such activity was evident only after the mycelium had penetrated to the parenchyma of the leaf. Death of the epidermal cells prior to the penetration of the cuticle was never found to occur.

In the case of the strain of *Botrytis cinerea* causing blossom and spur blight of pear, the stages of infection observed were rather distinct and worthy of mention here. When conidia were dusted on

[5] WARD, H. M. A LILY DISEASE. Ann. Bot. [London] 2: 319-382, illus. 1888.
[6] BLACKMAN, V. H., and WELSFORD, E. J. STUDIES IN THE PHYSIOLOGY OF PARASITISM. II. INFECTION BY BOTRYTIS CINEREA. Ann. Bot. [London] 30: 389-398, illus. 9116.

pear blossoms in the greenhouse, infection started in one of four places. In some flowers the stamens wilted first, with the infection apparently having its incipiency somewhere along the filament; invasion proceeded from the filament to the receptacle cup and thence to the neighboring petals, sepals, stamens, and styles, causing immediate browning and wilting. In other flowers styles were infected first, whereas in still others sepals were first infected. In a very few cases small bracts on the flower pedicels became infected first.

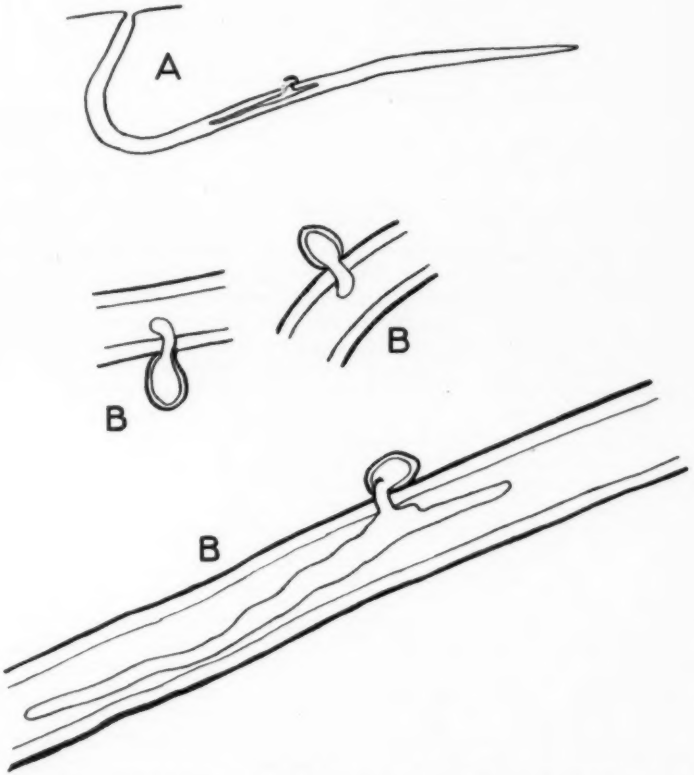

FIG. 5.—A, epidermal hair from the filament of a stamen of a pear blossom, showing the spore and germ tube after infection has taken place. × 115. B, camera-lucida diagrams of the spores and germ tubes in the incipient stage of infection of the epidermal hairs of stamens. × 720

Figure 4 shows several flowers in the early stages of blight. Figure 4, A, shows about one-half of the flower invaded. In Figure 4, B, the petals have been plucked away to show the single infected style. In Figure 4, C, two styles are attacked. Figure 4, D, shows two sides of the receptacle and all of the styles infected. Figure 4, E, shows two styles and one side of the cup infected. Figure 4, F, is an example of petal infection which has invaded a short distance into the receptacle.

Mycelial invasion of the tissues proceeds very rapidly. For instance, a stamen was found somewhat browned and beginning to wilt at 9 a. m. At 2.15 p. m. the invasion had spread until about one-fourth of the calyx disk was browned and one petal and two sepals were wilted.

Microscopic examination revealed that infection may take place by the conidia adhering to the epidermal hairs of the floral parts and the germ tubes entering directly through the wall of the hairs. This was observed in many cases, and no instance of mechanical pressure or peglike growth from a superficial germ tube was observed. After penetrating the wall of the hair the germ tube makes rapid growth toward the epidermal cell. Figure 5, A, shows a hair from the stamen of an Anjou flower. Figure 5, B, shows several early infections of hairs greatly enlarged.

There has been no opportunity for trials of sprays. The disease has not been continuously severe enough to warrant the growers to expend time and materials for sprays to control this disease alone.

SUMMARY

A spur and blossom blight of pears in Oregon is described. It attacks the Anjou, Bartlett, Winter Nelis, Comice, and Bosc in severity in the order mentioned. As far as the writer knows the disease is limited almost entirely to southwestern Oregon. As many as 15 per cent of fruiting spurs have been killed in the most severe cases found affecting Anjou pear. Conidia from sclerotia on hold-over lesions infect through the hairs of the floral parts.

THE RELATIVE UTILIZATION OF FEED ENERGY FOR MAINTENANCE, BODY INCREASE, AND MILK PRODUCTION OF CATTLE [1]

By E. B. FORBES, *Director*, J. AUGUST FRIES, *Assistant Director*, WINFRED W. BRAMAN, *Associate in Animal Nutrition*, and MAX KRISS, *Associate in Animal Nutrition, Institute of Animal Nutrition, Pennsylvania State College*

INTRODUCTION

In 1924 a paper [2] representing cooperative work by the Institute of Animal Nutrition of Pennsylvania State College and the United States Department of Agriculture was published, in which preliminary results on the relative utilization of energy in milk production and body increase of dairy cows were given. Further data of a generally similar but greatly improved character are now available, and these are given in the present report. In the earlier study the heat production and gains of energy were computed from the carbon and nitrogen balances, the maintenance requirement of energy was estimated from published averages, the net energy utilized for body gain was estimated from results with a single cow, and the computed heat production was not corrected to a standard day, as to standing and lying.

In the experiments upon which the present report is based several cows were used, the heat production and gains of energy were determined by direct calorimetry, the maintenance requirement of net energy was also directly observed with each cow, as the fasting katabolism, the net energy utilized for body gain was determined with each cow while she was dry, and a new procedure was utilized for computing the heat production to the standard day as to standing and lying.

The investigation herein reported comprises three series of experiments,[3] conducted during the years 1921, 1922, 1923; and it deals with the efficiency of the milk cow, in terms of energy, to utilize feeding stuffs, especially for milk production, in comparison with maintenance and body gain.

PLAN OF THE INVESTIGATION

The utilization of feed energy in milk production is here considered in relation to that portion of the feed which is available for milk production after deducting the nutritive requirements of maintenance and any such body gain as may have occurred. The milk energy is thus regarded as net energy utilized in the production of milk.

The schedule of the experiments involving milk production is set forth in Table 1. The first two experiments, 221E and 221F, were planned to cover two series of observations with each cow, one as early and one as late as practicable in the period of lactation, and on a plane of nutrition estimated to be slightly above the requirements

[1] Received for publication Apr. 1, 1926; issued September, 1926.

[2] FRIES, J. A., BRAMAN, W. W., and COCHRANE, D. C. RELATIVE UTILIZATION OF ENERGY IN MILK PRODUCTION AND BODY INCREASE OF DAIRY COWS. U. S. Dept. Agr. Bul. 1281, 36 pp., illus. 1924.

[3] Of these the first two were planned by the late H. P. Armsby, the third by J. A. Fries; and all were conducted under the immediate supervision of J. A. Fries, with the cooperation of W. W. Braman, D. C. Cochrane, Max Kriss, C. D. Jeffries, W. J. Sweeney, and R. M. Meredith.

for maintenance and milk production, but not permitting of fattening to a significant extent. The third experiment, 221G, was planned to determine, in conjunction with the study of utilization of feed energy, the effects upon milk production of different quantities of the same feed. This last experiment consisted of four periods with one cow on widely different quantities of the standard feed mixture. During the first and fourth periods the cow was given as much feed as she would eat without waste; in the second period the ration was as computed to equal the maintenance requirement; and in the third period the ration was increased by half of the difference between those of the first and the second periods.

TABLE 1.—*Experimental periods, rations, and live weights of experimental subjects*

Experiment No.	Cow No.	Period No.	Preliminary feeding period	Collection period	Rations		Feed refused (average per day)	Average live weights
					Alfalfa hay	Grain		
					Kgm.	*Kgm.*	*Kgm.*	*Kgm.*[a]
221E	874	I	Dec. 25–Jan. 4	Jan. 5–Jan. 14, 1921	3.140	4.700	None.	381
	874	II	Mar. 12–Mar. 22	Mar. 23–Apr. 1, 1921	3.040	4.560	None.	391
	886	I	Jan. 8–Jan. 18	Jan. 19–Jan. 28, 1921	3.700	5.540	None.	398
	886	II	Mar. 26–Apr. 5	Apr. 6–Apr. 15, 1921	3.200	4.800	None.	401
221F	886	I	Dec. 23–Jan. 3	Jan. 4–Jan. 13, 1922	4.370	6.560	[b]0.774	416
	886	II	Mar. 25–Apr. 4	Apr. 5–Apr. 14, 1922	3.660	5.490	None.	412
221G	887	I	Nov. 25–Dec. 5	Dec. 6–Dec. 15, 1922	3.651	5.476	None.	383
	887	II	Dec. 23–Jan. 2	Jan. 3–Jan. 12, 1923	1.750	2.620	None.	346
	887	III	Jan. 13–Jan. 23	Jan. 24–Feb. 2, 1923	2.700	4.050	None.	345
	887	IV	Feb. 3–Feb. 13	Feb. 14–Feb. 23, 1923	3.650	5.480	None.	358

[a] Average of eight daily weighings, taken before watering, at about 8 a. m., during the collection period.
[b] Average of 10 days. Of the entire amount refused, 3.31 kgm. were refused during the two days in which the calorimetric study was made.

Each experimental period covered 21 days, the first 11 being preliminary, and the last 10 the collection period. During the latter period the milk, urine, and feces were collected and sampled for chemical analysis; and during the last two days of each collection period the animal was subjected to a respiration calorimetric study in which heat production, carbon dioxide, water vapor, and methane were measured.

EXPERIMENTAL SUBJECTS

Three Jersey cows were used in these investigations. Two of them, Nos. 886 and 887, were purebred, and one, No. 874, was of unknown breeding. Cow 886 was born July 13, 1914. Her first calf was dropped October 10, 1917, her second calf November 28, 1920, and her last calf December 24, 1921. She was used dry in experiment 221D (1920), and fresh in experiment 221E and 221F (1921 and 1922). Cow 874 was born May 5, 1916. Her second calf was dropped July 27, 1920. She was used fresh in experiment 221E, and dry in experiment 221F. Cow 887 was born September 5, 1917. Her third calf was dropped October 27, 1922. She was used dry in experiment 221F, and in milk in experiment 221G (1923). These animals possessed the usual nervous temperament of Jersey cattle. No one was a hearty feeder, and all were rather slow in adapting themselves to the changes in ration. All were fair milkers, for their breed.

FEEDS AND RATIONS

The standard ration used throughout all experiments considered in this paper consisted of alfalfa hay of good quality cut into about 1-inch lengths, and a grain mixture composed of 10 per cent linseed meal, 30 per cent wheat bran, 30 per cent ground oats, and 30 per cent corn (maize) meal, all of good quality. The ratio of hay to grain as fed throughout all the experiments was as 2 to 3, by weight. Common salt was fed in the amount of 30 gm. per day, 15 gm. at each feeding

In consideration of the nature of the ration, and of its individual components, it was assumed that the amino acids of the protein mixture were in efficient variety, and that the mineral and vitamin requirements of the animals were satisfied.

On account of the disqualifying refusal of feed by cow No. 886, in Period I of experiment 221F, and other technical difficulties with cow No. 874, in Period II, experiment 221E, the data from these periods are excluded from this discussion.

METHODS OF EXPERIMENTATION

The rations were weighed in covered tin cans in which they were kept until needed; and the sampling was performed by a carefully considered procedure which need not be described in detail.

The urine and feces were collected together. At the end of each 24 hours these excreta were weighed, thoroughly mixed, and an aliquot weighed out for a composite sample. Another portion was weighed in a shallow tin pan, and dried at about 60° C. The composite sample was preserved in a zinc can by the addition of carbon bisulphide, and at the end of the 10-day collection period the composite sample was well mixed, and a portion dried and prepared for analysis, as were also the daily samples.

Samples of the milk were preserved by the addition of 1 c. c. of 40 per cent formalin per liter. From these daily samples aliquots were weighed out for the composites. These composite samples were preserved, in duplicate, and kept cool until the analyses were completed.

The milking was done at half past 4 o'clock, morning and evening. For this purpose the main door of the calorimeter was opened, thus permitting the milker to enter. Corrections for the heat, carbon dioxide, and water vapor given off by the milker while in the calorimeter were made on the basis of published data considered satisfactory for the purpose.

The feeds and dried excreta were analyzed by methods conforming in all essential details to the procedures of the Association of Official Agricultural Chemists;[4] the heat of combustion and the carbon being determined by means of a bomb calorimeter; and in the last two experiments carbon was also determined by means of a combustion furnace. Nitrogen was determined in the fresh excreta by the König method in all experiments.

[4] ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS, COMPILED BY THE COMMITTEE ON EDITING METHODS OF ANALYSIS. REVISED TO JULY 1, 1924. Ed. 2, 535 p., illus. Washington, D. C. 1925.

TABLE 2.—*Balance of matter and energy per day and head*

	Experiment 221E, Cow 874, Period I					Experiment 221E, Cow 886, Period I				
	Dry matter	Water	Nitrogen	Carbon	Energy	Dry matter	Water	Nitrogen	Carbon	Energy
Income:	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Cal.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Cal.*
Alfalfa hay	2,822.9	341.0	75.6	1,300.4	12,646.0	3,325.9	363.5	89.1	1,532.1	14,899.3
Mixed grain	4,055.8	639.0	106.2	1,886.1	18,816.0	4,779.3	713.0	125.1	2,222.6	22,172.5
Water		19,072.5					18,380.0			
Total	6,878.7	20,052.5	181.8	3,186.5	31,462.0	8,105.2	19,456.5	214.2	3,754.7	37,071.8
Outgo:										
Feces and urine	[a] 2,771.1	9,491.9	[b] 132.0	[a] 1,162.2	[a] [c] 11,773.5	[a] 3,186.5	13,055.4	[b] 152.0	[a] 1,344.1	[a] [c] 13,545.6
Methane	206.4			154.5	2,754.2	257.5			192.7	3,436.1
Milk	830.0	4,313.4	28.7	441.7	4,931.2	1,133.5	6,608.2	43.8	569.4	6,497.8
Hair and scurf	11.8	0.7	1.0	4.8	51.6	14.4	0.7	1.5	6.5	71.2
Carbon dioxide	5,031.1			1,372.0		5,466.4			1,490.7	
Water vapor		6,396.0					7,114.8			
Metabolizable:										
Income minus urine, feces, and methane					16,934.3					20,090.1
Body balances:										
Fat	−15.8			−12.1	−150.1	+128.1			+98.0	+1,217.0
Protein	+120.6		+20.1	+63.4	[a] +537.7	+101.4		+16.9	+53.3	[c] +452.1
Water		−149.5					−7,322.6			
Computed heat production					11,563.9					11,852.0

	Experiment 221E, Cow 886, Period II					Experiment 221F, Cow 886, Period II				
Income:										
Alfalfa hay	2,811.6	374.0	65.0	1,303.9	12,651.1	3,277.6	402.4	73.3	1,530.9	14,837.7
Mixed grain	4,178.3	514.5	108.9	1,953.5	19,550.0	4,789.3	698.2	123.7	2,239.6	22,308.9
Water		13,580.0					16,320.0			
Total	6,989.9	14,468.5	173.9	3,257.4	32,201.1	8,066.9	17,420.6	197.0	3,770.5	37,146.6
Outgo:										
Feces and urine	[a] 2,816.8	11,072.2	[b] 131.4	1,173.6	[a] [c] 11,881.5	[a] 3,259.0	13,373.5	[b] 151.3	[b] 1,360.4	[a] [c] 13,756.0
Methane	175.5			130.2	2,341.9	198.54			148.6	2,649.3
Milk	884.7	5,031.0	34.0	461.0	5,162.2	998.6	6,007.8	39.1	531.4	5,934.7
Hair and scurf	10.3	0.7	1.0	4.6	49.5	13.6	0.7	1.3	5.9	64.4
Carbon dioxide	4,948.1			1,349.3		5,572.95			1,519.7	
Water vapor		4,484.7					5,910.6			
Metabolizable:										
Income minus urine, feces, and methane					17,977.7					20,741.3
Body balances:										
Fat	+150.4			+115.1	+1,428.8	+245.5			+187.8	+2,332.3
Protein	+45.0		+7.5	+23.6	[a] +200.6	+31.8		+5.3	+16.7	[a] +141.8
Water		−6,120.1					−7,872.0			
Computed heat production					11,136.6					12,268.1

	Experiment 221G, Cow 887, Period I					Experiment 221G, Cow 887, Period II				
Income:										
Alfalfa hay	3,241.5	418.0	77.4	1,495.0	14,680.4	1,553.7	186.0	37.1	716.6	7,036.5
Mixed grain	4,791.7	668.0	122.1	2,231.2	22,530.5	2,292.6	318.0	58.4	1,067.5	10,779.8
Water		17,060.0					11,100.0			
Total	8,033.2	18,146.0	199.5	3,726.2	37,210.9	3,846.3	11,604.0	95.5	1,784.1	17,816.3
Outgo:										
Feces and urine	[a] 3,223.4	11,223.0	[b] 147.0	[b] 1,378.6	[a c] 13,873.1	[a] 1,734.5	6,818.7	[b] 93.1	[b] 718.8	[a c] 7,054.6
Methane	198.7			148.7	2,651.5	104.0			77.9	1,387.8
Milk	1,347.0	7,456.2	48.6	705.4	7,947.2	891.5	5,162.6	27.5	482.6	5,588.5
Hair and scurf	10.6	0.4	0.9	4.8	53.2	6.8	0.3	0.6	3.1	33.8
Carbon dioxide	5,452.6			1,486.9		3,450.7			941.0	
Water vapor		5,951.9					3,141.1			
Metabolizable:										
Income minus urine, feces, and methane					20,686.3					9,373.9
Body balances:										
Fat	−10.06			−7.7	−95.6	−468.3			−358.3	−4,448.9
Protein	+18.0		+3.0	+9.5	[c] +80.2	−154.2		−25.7	−81.0	[c] −687.4
Water		−6,485.5					−3,518.7			
Computed heat production					12,701.3					887.9

	Experiment 221G, Cow 887, Period III					Experiment 221G, Cow 887, Period IV				
Income:										
Alfalfa hay	2,383.4	296.0	60.4	1,092.3	10,759.2	3,222.0	448.0	81.6	1,476.6	14,544.8
Mixed grain	3,551.2	498.0	91.4	1,656.9	16,719.4	4,805.1	449.0	123.7	2,242.0	22,622.8
Water		15,725.0					19,735.0			
Total	5,934.6	16,519.0	151.8	2,749.2	27,478.6	8,027.1	20,632.0	205.3	3,718.6	37,167.6
Outgo:										
Feces and urine	[a] 2,402.1	10,452.9	[b] 110.3	[b] 1,014.3	[a c] 10,198.3	[a] 3,327.0	14,317.7	[b] 153.3	[b] 1,408.8	[a c] 14,276.6
Methane	153.7			115.0	2,051.0	194.5			145.6	2,595.4
Milk	951.8	5,945.3	33.4	505.2	5,647.8	1,049.1	6,308.8	40.5	543.0	6,044.5
Hair and scurf	7.0	0.3	0.6	3.2	35.0	9.9	0.9	0.9	4.5	49.5
Carbon dioxide	4,309.4			1,175.2		5,242.8			1,429.7	
Water vapor		4,200.0					6,336.4			
Metabolizable:										
Income minus urine, feces, and methane					15,229.3					20,295.6
Body balances:										
Fat	−114.1			−87.3	−1,084.0	+200.8			+153.6	+1,907.6
Protein	+45.0		+7.5	+23.6	[c] +200.6	+63.6		+10.6	+33.4	[c] +283.5
Water		−4,079.5					−6,331.8			
Computed heat production					10,429.9					12,010.5

[a] Corrected for loss on drying

[b] Determined on fresh substance.

[c] Corrected to nitrogen equilibrium.

In the air current entering the calorimeter the carbon dioxide and water vapor were determined gravimetrically in a continuous sample, and in the outcoming air the carbon dioxide, water vapor, and methane were likewise determined in a continuous sample. Determinations were also made of oxygen and of carbon dioxide, in the outcoming air current, by means of a Sondèn apparatus, on samples collected at half-hour intervals during the last 24 hours of each calorimeter period.

THE BALANCE OF MATTER AND ENERGY

The income, outgo, and balances of matter and energy for each cow and for each experimental period are recorded in Table 2. These foundation data are submitted for record only. The balance of energy was computed from the balances of protein and fat, by the use of the usual factors, the energy of the protein being corrected to nitrogen equilibrium, as was also the energy of the feces-and-urine mixture. It should be borne in mind that these energy balances rest upon a different experimental basis from those appearing in Table 3, which were obtained by the use of the directly observed heat production. This direct heat production and the computed heat production do not differ in any case to an important degree. The significance of these data will be discussed in the later, derived tables.

TABLE 3.—*Energy of milk produced and of body gain, based on the observed heat production*

Experiment No.	Cow No.	Period No.	Dry matter in feed eaten	Metabolizable energy		Observed heat production	Energy of milk	Gain of energy by body
				Per kgm. of dry matter of feed	Total			
			Kgm.	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*
221E	874	I	6.879	2,462	16,934	11,417	4,931	+586
	886	I	8.105	2,479	20,090	12,048	6,498	+1,544
		II	6.990	2,572	17,978	11,049	5,162	+1,767
221F	886	II	8.067	2,571	20,741	12,422	5,935	+2,384
221G	887	I	8.033	2,575	20,686	12,368	7,947	+371
		II	3.846	2,437	9,374	8,627	5,589	−4,842
		III	5.935	2,566	15,229	9,870	5,648	−289
		IV	8.027	2,528	20,296	11,670	6,045	+2,581

ENERGY OF MILK PRODUCED AND OF BODY GAIN

The metabolizable energy (gross energy minus energy of excreta) per kilogram of dry matter of feed (Table 3) agreed satisfactorily for the different cows and periods, indicating the existence of consistent experimental conditions. To explain the significance of this table, it should be stated that the total metabolizable energy, minus the observed heat production and the energy of the milk, equals the gain of energy by the body.

The observed heat production, the energy of the milk, and the energy of the body gain differed, from period to period, as determined by the differences in plane of nutrition. Since the cow tends strongly to maintain her individually characteristic milk production, regardless of variations in the feed, the radical changes in plane of nutrition affected the energy of the body gain much more prominently than they did the energy of the milk.

Attention is called to the fact that the observed heat production as recorded in Table 3 has not been corrected to the standard day, as to standing and lying; hence the balances of energy cover the individuality of the cows with regard to time and energy spent in these two positions.

Before the net-energy values of the feed for milk production can be computed it must first be known how much feed and metabolizable energy should be allowed for the maintenance of these animals, and for any gain of energy by the body which occurred.

The computation of these requirements (of feed and metabolizable energy) was based upon (1) the maintenance requirement of energy (heat production of fasting), (2) the net-energy value of the ration, and (3) the percentage utilization of the metabolizable energy of the ration for maintenance, the derivation of which data is as indicated in Table 4.

TABLE 4.—*Computation of the net-energy value of the ration and the utilization of the metabolizable energy in the maintenance of dry cows*

Experiment No.	Cow No.	Period No.	Body surface in maintenance periods	Fasting katabolism per square meter per day [a]	Total fasting katabolism	Heat production in maintenance periods per day [a]	Heat increment		Metabolizable energy per kilogram dry matter	Net energy per kilogram dry matter of feed	Utilization of metabolizable energy
							Total	Per kilogram dry matter of feed			
			Sq. m.	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Per cent*
221D	886	I	4.70	[b]1,331	6,256	8,085	1,829	480	2,429	1,949	80.2
		III	4.84	[b]1,331	6,442	8,026	1,584	419	2,476	2,057	83.1
Average					6,349			450	2,453	2,003	81.7
221F	874	II	4.81	[c]1,446	6,955	9,093	2,138	534	2,397	1,863	77.7
	887	II	4.08	[c]1,483	6,051	8,274	2,223	613	2,484	1,871	75.3

[a] Twelve hours standing and 12 hours lying.
[b] Results of the third day of fast.
[c] Average of days of fast 6½ to 7½ and 7½ to 8½.

The fasting katabolism of cow 886 (fifth column of Table 4) is for the third day of fast, whereas in the case of cows 874 and 887 the data represent the average of days 6½ to 7½ and 7½ to 8½ of fast. It will be noted that the total fasting katabolism per day has been computed according to the estimated body surface of the animals in the maintenance periods, so as to render the metabolism in the fasting and maintenance periods comparable in respect to surface area.

It should be understood that the methods followed in this computation are provisional only, and that the determination of the fasting katabolism as a measure of the maintenance requirement of energy has yet to be standardized.

The difference between the heat produced per day in the maintenance periods and the heat produced per day while fasting is the heat increment, or energy cost of feed utilization. Subtracting the heat increment per kilogram of dry matter of the ration from the metabolizable energy per kilogram of dry matter in the maintenance periods gives the net energy value of the feed, per kilogram of dry matter, for maintenance. The values so derived are contained in the next to the last column of Table 4. In the last column of this table are found the percentages of utilization of the metabolizable energy, obtained by dividing the net energy by the metabolizable energy, per kilogram of dry matter.

The data of Table 4, as already stated, are made the basis for computing the dry matter of feed required for maintenance and the requirement of metabolizable energy for the milking cows. These requirements have been computed as indicated in Table 5.

TABLE 5.—*Maintenance requirement of net and of metabolizable energy and of dry matter of feed for the milking cows*

Experiment No.	Cow No.	Period No.	Maintenance requirement of net energy	Correction for standing	Maintenance requirement of net energy, corrected for standing	Net energy values for maintenance, per kilogram dry matter of feed	Utilization of metabolizable energy	Dry matter of feed required for maintenance	Metabolizable energy required for maintenance
			Cals.	*Cals.*	*Cals.*	*Cals.*	*Per cent*	*Kgm.*	*Cals.*
221E	874	I	6,955	+154	7,109	1,863	77.7	3.816	9,149
	886	I	6,349	+75	6,424	2,003	81.7	3.207	7,863
		II	6,349	+99	6,448	2,003	81.7	3.219	7,892
221F	886	II	6,349	+25	6,374	2,003	81.7	3.182	7,802
221G	887	I	6,051	+179	6,230	1,871	75.3	3.330	8,274
		II	6,051	+172	6,223	1,871	75.3	3.326	8,264
		III	6,051	+205	6,256	1,871	75.3	3.344	8,308
		IV	6,051	+101	6,152	1,871	75.3	3.288	8,170

The allowances for body gain have been computed on the basis of the observed gains and losses (as indicated in the last column of Table 3) and the corresponding net-energy values of the feed, those for body gain having been determined elsewhere from results of supermaintenance feeding.[5]

TABLE 6.—*Feed available for milk production and milk energy per kilogram of dry matter of available feed*

Experiment No.	Cow No.	Period No.	Body gain or loss	Net energy per kilogram dry matter for body gain or loss	Dry matter of feed required for body gain or loss	Dry matter of feed required for maintenance	Dry matter of feed available for milk production	Milk energy	
								Total	Per kilogram of available feed
			Cals.	*Cals.*	*Kgm.*	*Kgm.*	*Kgm.*	*Cals.*	*Cals.*
221E	874	I	+586	1,520	0.386	3.816	2.677	4,931	1,842
	886	I	+1,544	1,396	1.106	3.207	3.792	6,498	1,714
		II	+1,767	1,396	1.266	3.219	2.505	5,162	2,061
221F	886	II	+2,384	1,396	1.708	3.182	3.177	5,935	1,868
Average (Cow No. 886)									1,881
221G	887	I	+371	1,438	.258	3.330	4.445	7,947	1,788
		II	−4,842	1,871	2.588	3.326	3.108	5,589	1,798
		III	−289	1,871	.154	3.344	2.745	5,648	2,058
		IV	+2,581	1,438	1.795	3.288	2.944	6,045	2,053
Average (Cow No. 887)									1,924

In case of a loss of energy by the body, the amount of feed required to prevent this loss was computed by the use of the net-energy value for maintenance given in Table 4. The results in detail are presented in Table 6, which gives also the dry matter of feed available for milk production and the energy of milk produced per kilogram dry matter

[5] COCHRANE, D. C., FRIES, J. A., and BRAMAN, W. W. THE MAINTENANCE REQUIREMENT OF DRY COWS. Jour. Agr. Research (1925) 31: 1055-1082. 1926.

of available feed (last column). The latter data represent the net-energy values per kilogram dry matter of the ration for milk production.

In Table 7 the metabolizable energy available for milk production, and the percentages of utilization of the metabolizable energy in milk production, have been computed in a similar manner.

TABLE 7.—*The utilization of metabolizable energy for milk production*

Experiment No.	Cow No.	Period No.	Body gain or loss	Utilization of metabolizable energy	Metabolizable energy required for body gain or loss	Metabolizable energy required for maintenance	Metabolizable energy available for production of milk	Milk energy	Utilization of metabolizable energy for milk production
			Cals.	*Per cent*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Per cent*
221E	874	I	+586	61.6	951	9,149	6,834	4,931	72.2
	886	I	+1,544	56.1	2,752	7,863	9,475	6,498	68.6
		II	+1,767	56.1	3,150	7,892	6,936	5,162	74.4
221F		II	+2,384	56.1	4,250	7,802	8,689	5,935	68.3
Average (cow No. 886)									70.4
221G	887	I	+371	57.0	651	8,274	11,761	7,947	67.6
		II	−4,842	75.3	−6,430	8,264	7,540	5,589	74.1
		III	−289	75.3	−384	8,308	7,305	5,648	77.3
		IV	+2,581	57.0	4,528	8,170	7,598	6,045	79.6
Average (cow No. 887)									74.7

The data of Tables 6 and 7 indicate a considerably greater efficiency of utilization of feed energy for milk production than for body production, and seem to show a tendency toward a more efficient utilization of the available feed energy in the later stages of lactation.

The average amounts of milk energy produced per kilogram dry matter of available feed are 1,842 Calories for cow 874, 1,881 Calories for cow 886, and 1,924 Calories for cow 887. The average percentages of utilization of the metabolizable energy for milk production are 72.2 per cent, 70.4 per cent, and 74.7 per cent, for cows 874, 886, and 887, respectively.

The average net energy values of the ration used for maintenance, body increase, and milk production, as obtained with these cows, have been assembled in Table 8 to illustrate the relative utilization of the feed energy for the three purposes mentioned.

TABLE 8.—*Relative value of feed energy for maintenance, body increase, and milk production*

Cow No.	Net energy value per kilogram of dry matter of feed			Utilization of feed energy		
	For maintenance	For body increase	For milk production	For maintenance (standard)	For body increase (relation to maintenance)	For milk production (relation to maintenance)
	Cals.	*Cals.*	*Cals.*			
874	1,863	1,520	1,842	1	0.816	0.989
886	2,003	1,396	1,881	1	.697	.939
887	1,871	1,438	1,924	1	.769	.1028
Average	1,912	1,451	1,882	1	.761	.985

Regarding the net energy value of the ration for maintenance as the standard of comparison—that is, as unity—and relating to this, respectively, the net-energy values for body increase and for milk production, we find the ratios of utilization of the feed energy as recorded in the last three columns of Table 8.

CONCLUSIONS

In a series of respiration calorimeter studies of the energy metabolism of cows, both in dry condition and in lactation, and on different planes of nutrition, the average rates of utilization of the net energy of the ration for maintenance, lactation, and body increase were found to be as 1 for maintenance, 0.985 for lactation, and 0.761 for body increase. More extensive evidence will doubtless modify this apparent relationship; but accepting the same provisionally, it is certainly of interest, and consistent, from a teleological point of view, that with a lactating female the rates of efficiency of utilization of food for the maintenance of the life of the mother and for the production of milk for the offspring are apparently alike, while the economy of use for body growth is at a distinctly lower rate.

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